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Papers from a workshop held at the NASA Ames Research Center Moffett Field, California July 16 - 19, 1985

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Controlled Ecological Life Support Systems: CELSS '85 Workshop

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January 1986

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National Aeronautics and
Space Administration

Ames Research Center
Moffett Field, California 94035

Papers from a workshop held at
the NASA Ames Research Center
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FOREWORD

This volume contains the scientific and technical papers presented at the Workshop on Controlled Ecological Life Support Systems, CELSS '85. Formal presentations occurred at the annual meeting of the Intersociety Conference on Environmental Systems (ICES) in San Francisco on July 17, 1985, and at NASA-Ames Research Center on July 18-19, 1985. The presentations at NASA-Ames were made in four topical sessions, which generally cover the diverse research areas necessary to achieve an operational CELSS. The Workshop sessions included: Plant Growth Chambers, Whole System Recycling and Control, Microbial Systems, and Plant Physiology.

The purpose of CELSS is to improve the environment for, and reduce the cost of, long-term human habitation in space. The intention in developing this Workshop was to bring together interested scientists and engineers, including those representing national space agencies other than NASA, to discuss the research and development of the technologies required for a CELSS. We eagerly welcomed the participation of the assembled scientists, engineers and visitors in this Workshop to share with us the excitement of planning for the future permanent presence of humans in space.

Robert D. MacElroy
Ames CELSS Program Manager
Planetary Biology Branch
NASA-Ames Research Center

WELCOME

William F. Ballhaus, Jr.

Director, NASA-Ames Research Center

It is my pleasure to welcome you to the CELSS '85 meeting on behalf of Ames Research Center. Ames is known primarily for its work in aeronautics, however about one-third of our effort is in space and life sciences. Even though the skyline of Ames is dominated by the aeronautics facilities, there is a tremendous amount of good space and life science research coming out of our laboratories. We also cooperate with a number of universities, with industry, and with other countries in our scientific research.

I would like to remind you of some of the things we have done in the past. The Pioneer series of spacecraft were all managed out of the Ames Research Center, and in fact Pioneer 6 is still the oldest operating U.S. spacecraft, 20-years-old this year, and it is still returning data. Pioneer 10 was the first man-made object to leave the known solar system. More significantly it was the first spacecraft to Jupiter. Pioneer 11 was the first spacecraft to Saturn and is now travelling out of the solar system in the opposite direction from Pioneer 10. Pioneer Venus is still taking data in orbit around the planet Venus, and will be used early next year for the Comet Halley studies. Other space science projects include the Galileo Probe, which was managed from Ames and just recently delivered to the Jet Propulsion Laboratory. The probe will be launched next year for an encounter with the Jovian atmosphere late in 1988. The Infrared Astronomical Satellite (IRAS) Telescope development was also managed by Ames Research Center.

In the area of aeronautics design and testing, we have the most complete set of wind tunnels and arc jets in the country as well as an extensive complement of flight simulators for both research and development. Ames has one of the most powerful computer facilities in the world with the Cyber 205 and two Cray XMP's. This autumn we plan to take delivery of the first large-memory Cray 2.

Today's symposium focuses on cooperation in biological science research. Since NASA's beginnings, Ames has been the primary center for basic research to study physiology, biotechnology, ecology and evolution of life. We believe there is a tremendous opportunity for international cooperation in that arena. CELSS is a long-term NASA effort, of course. The long-term goal is a sustained presence of humans in space, and the development of new life support systems will happen before this goal becomes a reality. But on the road to that objective there are many important scientific questions to be answered. For example, how is plant growth affected by different environments; how is long-term stability maintained in life support systems that have biological components; and how productive can organisms be under ideal, controlled circumstances? All very interesting, key questions in the establishment of a CELSS. You have my best wishes for a productive and entertaining meeting.

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INTRODUCTION

The engineering sophistication gained thus far in the manned space programs of the U.S., U.S.S.R. and the European Space Agency makes it possible for humans to plan for a permanent presence in space, possibly the next evolutionary step for our species. Life support systems provide the environmental control in sealed spacecraft that makes it possible for humans, not only to survive, but to work on complex tasks in the inhospitable environment of space. Space technology will eventually support establishment of long-term habitats on the Moon, and a chance for humans to set foot for the first time in a potentially fascinating environment, that of Mars.

One of the most interesting aspects of recycling, or regenerating, life support supplies in space is the reduction in cost compared to the alternative of continuously launching these supplies from Earth. The larger the crew and the longer the mission duration, the greater the potential for cost savings. Most of the short-term spaceflights to date have rarely used regenerative technologies. However, with the increase in the yearly number of expeditions (as with the U.S. Shuttle program), and an increase in mission duration (as with the U.S.S.R. Salyut space station), regenerative systems become increasingly attractive.

Chemical regenerative systems that reuse spent air and water have been studied by both the Soviet Union and the United States. Much of the work in the U.S. was done in the 1950's and 60's in the Aerospace Medicine laboratories of the Air Force, and at NASA-Langley Research Center. NASA-Ames Research Center became involved with the studies during the 70's. At this time many laboratories in many countries are involved in regenerative life support investigations. Researchers have reported their progress at the CELSS '85 Workshop at Ames Research Center, and their papers are included in this volume.

Bioregenerative systems, such as a CELSS, use living organisms to achieve revitalization of life support materials: removal of carbon dioxide, production of oxygen, and purification of water. In contrast to physical-chemical regenerative systems, the photosynthetic organisms in a CELSS also produce the basic foods essential for the maintenance of human life, allowing the evolution of an increasingly self-sustaining permanent human habitation beyond the Earth. Studies of plant growth in controlled environment chambers have been conducted in the Soviet Union, in France, and more recently in the U.S. at NASA-Ames Research Center in California. In the immediate future, a new U.S. CELSS project at NASA-Kennedy Space Center will develop a large plant growth chamber, approaching Space Station volumes.

The successful development of a CELSS for spaceflight will demand a deeper level of understanding of biological and agricultural science. The concept of the CELSS program is a vertical one, ranging from fundamental science to the flight of a life support system. The program thus envisions a sequence that requires that fundamental science be applied, that applied science be transferred to the realm of technology development, and that the technology be made space-worthy. The job is large and complex, and would benefit greatly from closer interactions between scientists and engineers, as well as closer cooperation among the international community of scientists.

This document was produced from the CELSS'85 series of meetings held in July of 1985. The Symposium on Scientific Cooperation in Biological Space Research was held at Ames; the session on International CELSS was held, under the auspices of the Society of Automotive Engineers, in San Francisco; and the sessions of the CELSS Investigators Meeting were held at Ames. The papers presented at these sessions are included in this volume, which also contains a brief summary of the remarks made by speakers at the Symposium on Scientific Cooperation.

SCIENTIFIC COOPERATION IN BIOLOGICAL SPACE RESEARCH

SYMPOSIUM SUMMARY
SCIENTIFIC COOPERATION IN BIOLOGICAL SPACE RESEARCH

The four-day CELSS'85 Workshop commenced with a day of lectures covering scientific cooperation in biological space research. These presentations were intended to recount our current level of understanding achieved from space life science research, and to encourage the international scientific community to work cooperatively in exploring biological issues associated with space research. A distinguished group of scientists covered a wide variety of topics all clustered about this central theme. What follows is a brief review of those remarks supplemented by a historical review of international efforts in CELSS related research. The entire symposium was videotaped and is available for viewing at NASA Ames Research Center.

Symposium Speakers

Dr. Harold P. Klein, Santa Clara University
- NASA's involvement in biological space research

Mr. John Sakss, International Affairs Division, NASA HQ
- International Cooperation in Space Research

Mr. Laurance A. Milov, Space Commercialization/Space Station Office, NASA Ames
- Space Commercialization Opportunities

Dr. James H. Bredt, Life Science Division, NASA HQ
- Applications of Bioregenerative Life Support

Dr. Richard Olson, Boeing Aerospace
- Space Station Configurations

Dr. Richard S. Young, MATSCO
- Space Station Research Opportunities

Dr. Wendell Mendell, Johnson Space Center
- Concepts for a Lunar Base

Comments by Representatives of Other National Organizations

Dr. David Cove, Science and Engineering Research Council, UK

Dr. Brad Thompson, Alberta Research Council, Canada

Dr. Richard Boudreault, Canadian Astronautics Ltd., Canada

Dr. Keiji Nitta, National Aerospace Laboratories, Japan

Dr. Å. Ingemar Skoog, Dornier Systems Gmbh., FDR

Dr. Alain Sarasin, Centre National Recherche Scientifique, France

Dr. Harold P. Klein, former Director of Life Sciences at Ames Research Center opened the day with a talk on NASA's Involvement in Biological Space Research. Dr. Klein's remarks covered a wide range of topics which reflected and summarized the various levels of NASA-sponsored research in biology: from the evolution of biogenic (literally "life-forming") elements

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within stars, through the prebiotic evolution of organic compounds and the evolution of cellular processes, through the multimillion-year time-scale of evolution on Earth (including possible solar system events that affected terrestrial life), through the effects of gravity on the evolution of organisms and their adaptation when these effects are reduced in space travel, and finally to understanding the global conditions necessary for life on Earth and assessing similar conditions on Mars and other solar system bodies.

Mr. John Sakss of the International Affairs Division of NASA Headquarters spoke on International Cooperation in Space Research. He discussed NASA's chartered role to achieve international cooperation and outlined the policies and practices of the Agency in accomplishing those mandates. He qouted President Reagan from a report to Congress in 1981 "...the scope and significance of international cooperation in space science are clear and visible." This talk emphasized the expansion of capabilities and confidence among NASA's traditional cooperative partners. Mr. Sakss mentioned the Soyuz-Apollo test project, the Space Shuttle and the Space Station as examples of successful cooperative ventures and expressed confidence that such joint endeavors would continue.

Mr. Laurance Milov of the Space Commercializaton/Space Station Office at NASA Ames talked about Space Commercialization Opportunities. His remarks were concerned with the role that NASA will play in opening space to commercial ventures and how the international community could be a part of that endeavor. Mr. Milov gave examples of earlier spinoffs from space related research and of current products under development such as pharmaceuticals and latex microspheres. The conclusion of these remarks emphasized the tremendous potential of space commercialization and the need for a firm commitment to it.

Dr. James Bredt of NASA Headquarters' Life Science Division talked about Applications of Bioregenerative Life Support. His comments covered the uses of CELSS as an operational spaceflight system, and focussed on the efficiency and functionality of the components that constitute the entire system.

Dr. Richard Olson of Boeing Aerospace directed his comments towards Space Station Configurations, which could include the addition of an experimental CELSS at some time after the assembly of the initial Space Station configuration. He discussed the many possibilities considered for design of the Space Station and spoke of the contributions being made by Europe and Japan.

Dr. Richard Young's remarks were directed at Space Station Research Opportunities. He described selected space life sciences experiments that have been conducted in the past, and that are being conducted on Shuttle flights. He also described the use of a CELSS as an essential component of manned planetary and lunar exploration.

Dr. Wendell Mendell of the Johnson Space Center spoke on Concepts for a Lunar Base. His remarks covered the wide range of designs and potential uses of a Lunar Base, including the need for international cooperation in the realization of such a facility. He noted that a return to the moon would be a fraction of the cost of the Apollo program, and while funding will not be needed until the 1990's, a national commitment to the moon as a long-term goal is needed for successful planning. Dr. Mendell directed the second part of his remarks toward

a Mars Base, indicating that many of the same principles of international cooperation were applicable to both Mars and Lunar bases.

At the end of the scheduled presentations time was left for comments by representatives of other National organizations. The many international visitors took advantage of the opportunity to make brief comments about the state of biological space research in their respective nations. Representatives of the United Kingdom, Canada, Japan, West Germany and France all spoke of the need to expand international efforts in space research. A common theme was that funding levels were not adequate to address the wide variety of research that is necessary. All indicated a desire to improve mechanisms for joint research and the exchange of information. The overall tone of the meeting was optimistic although the difficulties of achieving a strong, continuous commitment to cooperative biological space research was recognized.

INTERNATIONAL CELSS

Drs. Robert D. MacElroy and
James H. Bredt, Chairmen

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INTERNATIONAL CELSS

The editors would like to thank the Society of Automotive Engineers (SAE) and particularly Antenor R. Willems, Director of SAE Publications Group, for permission to reprint the papers that follow. They were prepared for the session entitled "International CELSS", and presented at the 15th Annual Intersociety Conference on Environmental Systems (ICES) on July 17, 1985 at the Cathedral Hill Hotel in San Francisco. This session included reports from scientists representing the U.S.A., France, Iran, Japan, and West Germany.

SAE takes a leading role in the execution of the annual ICES, which is jointly sponsored by SAE and four other technical societies: American Society of Mechanical Engineers (ASME), American Institute of Aeronautics and Astronautics (AIAA), American Institute of Chemical Engineers (AIChE), and the Aerospace Medical Association (ASMA). The ICES program includes a broad spectrum of topics ranging from aerospace thermal control systems to life support systems, and from space medicine and life science research to extra-vehicular activity (space walk) operations. The session on International CELSS was organized by Dr. Robert D. MacElroy, CELSS Program Manager at NASA-Ames Research Center; by Dr. James H. Bredt, Discipline Scientist for Biological System Research, NASA Headquarters; by Dr. Richard Olson of the AIAA's Life Sciences and Systems Technical Committee; and with organizational, technical and editorial support from Norman V. Martello and David T. Smernoff.

It is noteworthy that the keynote address at this 15th ICES was dedicated to the memory of Phillip D. Quattrone of NASA-Ames Research Center, who passed away suddenly in December 1984. He was a founding organizer of the ICES and was responsible for the development of prototype air and water regeneration systems, which will be used on Space Station. Although bioregenerative life support research was discontinued in within NASA in the late 1960's, Phil was pivotal in revitalizing the program again in 1979. His tireless efforts in the promotion and study of advanced life support and CELSS represents the foundation on which is based much of the work that we pursue today.

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Potential for Utilization of Algal Biomass for Components of the Diet in CELSS

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Z. Nakhost,
and M. Karel

Dept. of Applied Biological Sciences
Massachusetts Institute of Technology

ABSTRACT

The major nutritional components of the green algae (Scenedesmus obliquus) grown in a Constant Cell Density Apparatus were determined. Suitable methodology to prepare proteins from which three major undesirable components of these cells (i.e., cell walls, nucleic acids, and pigments) were either removed or substantially reduced was developed. Results showed that processing of green algae to protein isolate enhances its potential nutritional and organoleptic acceptability as a diet component in Controlled Ecological Life Support System.

The challenge of producing safe, nutritious, and acceptable fabricated foods in a space habitat (Controlled Ecological Life Support System = CELSS) requires conversion of algal biomass cells into protein (SCP = Single Cell Protein) as a possible food component (Karel, 1980, Karel and Kamarei, 1984). The role of algae in CELSS is rather unique. High photosynthetic activity and lack of need for "organic" carbon or nitrogen substrate, make algae systems suitable candidates for a primary oxygen regenerating system within CELSS (Lachance, 1968, Waslien et al., 1978, Clement, 1975; Santillan, 1982, Casey and Lubitz, 1963). Other possible algal functions include water purification, waste processing, nitrogen fixation, and removal of volatile gases. Cultivation of algae for above purposes will result in large quantity generation of algae biomass from which nutrients can be isolated and utilized as food component for the space crew.

Nutritional and safety considerations of SCP consumption have been addressed to a limited extent by Omstedt et al. (1973); Bourges et al. (1971), Mitsuda (1973), Kraut et al. (1966), and recently by Tuse (1984). There are 3 genera of algae (Scenedesmus, Spirulina, and Chlorella) with relatively known nutritional safety record. However consumption of unrefined algae by humans may cause side effects such as gastrointestinal disorders and allergic responses (Waslien, 1975).

The variations in the reported nutrient content of algae reflects to a great extent the composition and environmental conditions of the cultivation media (Clement et al., 1967; Lipinsky and Litchfield, 1970; Waslien, 1975; Becker, 1981; Santillan, 1982; and Piorreck et al., 1984).

Review of the literature also shows that while the proteins are the major component of the three mentioned algae genera the nutrition value of the "whole algae" is far less than animal proteins such as egg and milk (Becher, 1981; Chen and Peppler, 1978).

Direct consumption of single cell biomass, without purification, is not feasible in the amount which would be of any significance to biomass recycling. This is because of physiological concerns (e.g. nondigestive components of cell wall and disturbing carbohydrates, excess nucleic acids, unknown toxins and allergens, etc.) as well as organoleptic concerns (unpleasant flavor, color, and texture). To utilize the generated algal biomass, as a food component in space, one should first overcome the physiological and organoleptic concerns due to undesirable or potentially undesirable components in the algae system. To accomplish this goal and to enhance the nutritional value and acceptability of fabricated foods containing algae, attempts were made to recover the algae proteins in a relatively pure form. Major reviews of work on algae for human consumption either on earth or in space, stress the need for

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developing appropriate food technology for this raw material, and all acknowledge the complete absence of an adequate existing knowledge base for this purpose (Waslien et al., 1978, Lachance, 1968;Rha et al.,1975;Litchfield,1977;Cooney et al., 1980). For this purpose, we developed a methodology to eliminate or largely reduce three known undesirable components of algae system (cell walls, nucleic acids, and pigments and lipids) and obtained "algae protein isolate" with a reasonably high yield.

MATERIALS AND METHODS

SOURCE OF ALGAE - Green algae (Scenedesmus obliquus) were grown in the Constant Cell Density Apparatus (CCDA) at Martin Marietta Lab (Baltimore, MD). In this continuous culture apparatus, photosynthetic parameters can be constantly controlled and monitored and algae can be harvested on demand.

Algae were grown at 32°C and pH 7.0 up to cell density of 0.55 mg (dry weight) per milliliter. The source of nitrogen was KNO_3 (2.0g/l) in the growth media and the source of carbon was 2% CO_2 in air. To harvest the algae, overflow from the culturing apparatus was collected in 4-liter containers (4°C). When the cells settled down, the supernatant was discarded and the remaining concentrated cell suspension was transferred to one liter bottles and centrifuged (7000 g) for 30 minutes. The pellet ("packed cells") was washed 3-4 times with distilled water to remove traces of growth media.

CELL WALL RUPTURE - To study the cell components and to isolate the algal proteins, the first task was to break the cell wall of the intact algae packed cells (fraction #1). For this purpose, we tried three physical methods namely: shear of freeze dried algae cells (using krups type 203 coffee mill, up to 8 min at room temperature), sonication of 3% algae solution (Branson sonifier cell disruptor 200, up to 24 min at 0°C), and homogenization of 7.6% algae solution (Sorvall omni-mixer, with 40% 450-500 µm glass beads, 16,000 RPM, up to 30 min at 0°C). Cell wall rupture was followed by direct microscopic inspection of the resultant solution.

Upon freeze drying of above solution (Vitrus Co., Gardiner, NY), we obtained algal flour (fraction #2) which was used, for determination of major nutritional composition.

COMPOSITION DETERMINATION - All composition determinations were done on duplicate samples and calculations of recovery (% yield) of various algae fractions were based on the initial weight of the algae flour (freeze dried broken cells). Moisture and ash determination were carried out according to A.O.A.C. (1980) methods.

For protein determination, nitrogen was determined according to the modified Kjeldahl method (A.O.A.C., 1980) as summarized in fig. 1. Protein concentration was then calculated by multiplying total nitrogen content by 6.25.

Total nitrogen also includes the nitrogen from nucleic acids as the prime interfering source. Therefore, we measured initially the total nucleic acid (RNA & DNA) concentration in various fractions and then subtracted these values from the total protein to obtain the actual protein concentration. Knowledge of nucleic acid concentration was also important for reduction of these undesirable constituents in the final products. Determination of Deoxyribo Nucleic Acid (DNA) and Ribo Nucleic Acid (RNA) in various fractions was accomplished by modified procedure of Schmidt and Thannhauser (1945) and similar to those described by Smillie and Krotkov (1960).

For this purpose each algal fraction (20 mg for RNA and 40 mg for DNA) was initially extracted with cold methanol (10 ml, 3x), centrifuged (10,000 RPM, 4 min, 4°C), extracted with cold 5% TCA (10 ml, ?x), centrifuged, and finally extracted with cold and then boiling ethanol (10 ml) to produce "algae residue". The algae residue was dried (37°C), powdered, and used for determination of DNA and RNA as summarized in fig. 2A and 2B.

Purified DNA (from calf thymus, Sigma, St. Louis, MO) and RNA (from bakers yeast, Sigma, St. Louis, MO) were treated similarly to "algae residue" for preparation of standards. For preparation of diphenylamine reagent, 1 gram of purified diphenylamine (recrystallized from boiling hexane to a white crystalline product) was dissolved in 100 ml glacial acetic acid and 2.75 ml of sulfuric acid. For preparation of Orcinol reagent, 1 gram of purified orcinol (dissolved in boiling benzene, decolorized with charcoal, and crystallized to a perfectly white crystalline product) was dissolved in 100ml HCl containing 0.5 g $FeCl_3$.

Total pigments and lipids were determined by extraction with boiling ethanol in a Soxhelt apparatus as summarized in fig. 3. The obtained values, under above conditions, were assumed to be the highest extractable values and were used as reference for extraction treatments. For routine extraction of pigments and lipids, we, however, submerged thimbles containing 200 mg algae protein concentrate in 75 ml absolute ethanol at various temperatures (20, 0, and -20°C). This solution was gently mixed until equilibrium was reached (usually overnight). Solvent could then be decolorized (within 1-2 hours) with 10 g charcoal at the same temperatures.

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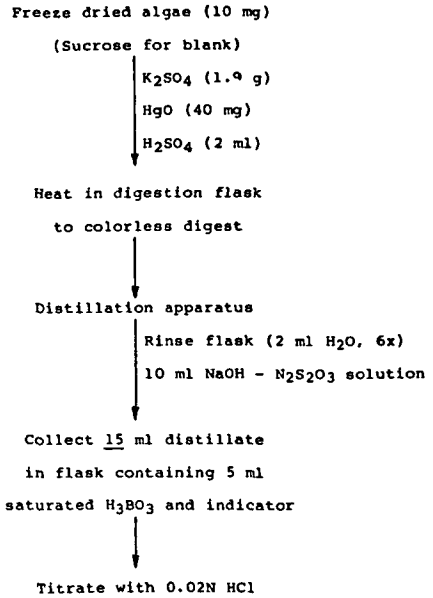

Fig. 1) Modified microkjeldahl procedure for "N" determination in various algae fractions. %N = (ml HCl - ml blank) x normality x 14.007 x 100/mg freeze dried algae.

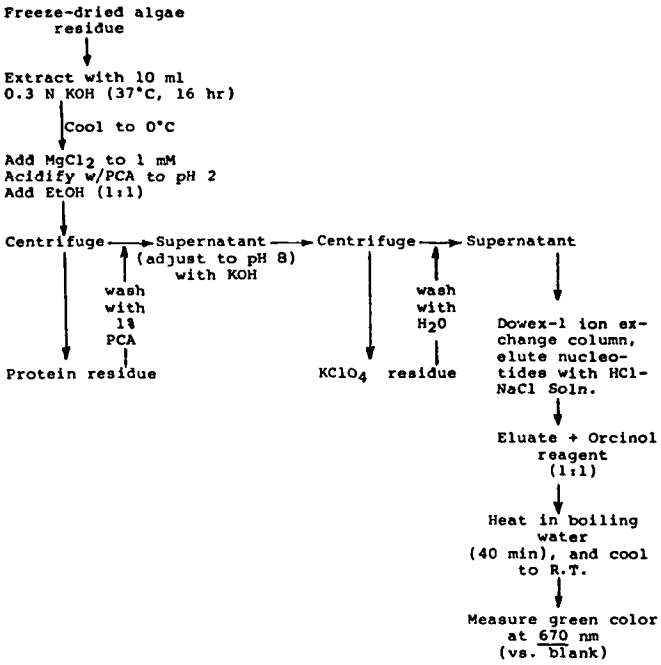

Fig 2B) The determination procedure for "RNA" in various algae fractions.

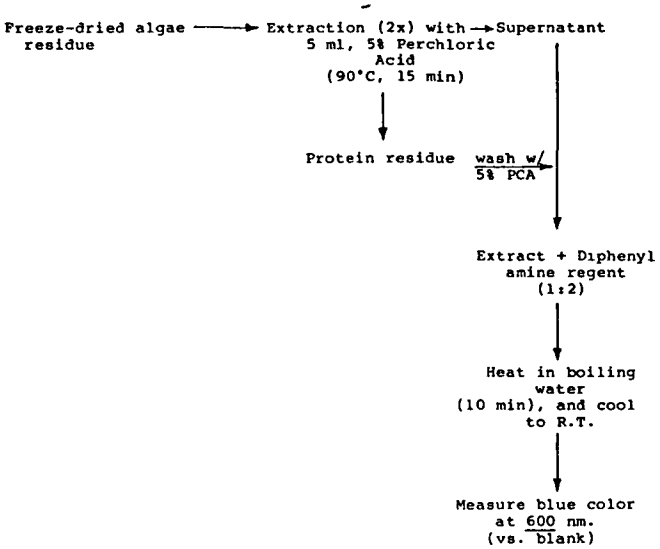

Fig 2A) the determination procedure for "DNA" in various algae fractions

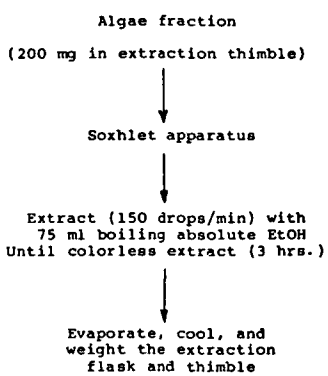

Fig 3) The determination procedure for total pigments and lipids in various algae fractions. % total pigments and lipids = wt. of extract (mg) x 100/wt. of algae (mg).

Total carbohydrates were calculated from the weight difference of all above components with that of the total original weight.

NUCLEIC ACID REDUCTION - Considering the overall conditions and limitations of space habitat we decided to use an enzymatic method for reduction of RNA and DNA, using extracellular RNase and DNase (both from bovine pancreas, Sigma, St. Louis, MO). To obtain the optimum reaction conditions for enzymatic treatment of algal nucleic acids, initially pure RNA and DNA were treated with RNase and DNase according to the method of Worthington (Millipore Corp., Bedford, MA) and under the following conditions:

For RNase treatment: 1 ml of 1% RNA in 0.1M sodium acetate (pH 5.0 and/or 7.4) was mixed with 1 ml of RNase in 0.1 M sodium acetate at concentrations of 0, 2.5, 10 and 50 g/ml. Upon incubation of the mixture at 37°C for 0, 4, and 8 min, the reaction was stopped with 1 ml of 25% PCA and ice bath (5 min). Following the centrifugation (5000 RPM, 10 min, 4°C) of the cold mixture, 0.1 ml of the supernatant was mixed with 2.9 ml water and increase in absorption at 260 nm, due to liberation of mono and oligo nucleotides, was measured.

For DNase treatment: 2.5 ml of 0.004% DNA (in 200 ml 6.25 mM $MgSO_4$, 25 ml 1.0 M sodium acetate, and 25 ml H_2O) at pH 5.0 and/or 7.4 was mixed with 0.5 ml DNase in 0.15 M NaCl at concentrations of 0, 25, 50, 100, and 200 µg/ml. Upon incubation of the mixture at 25°C for 0, 5, and 10 min, the reaction was stopped with 1.5 ml 25% PCA and ice bath (5 min). Following centrifugation (5000 RPM, 10 min, 4°C) of the cold mixture, absorption at 260 nm was determined. Based on the obtained optimum conditions for enzymatic treatment of "pure" RNA and DNA and with a similar treatment, freshly-prepared (i.e., non freeze-dried) algae crude protein (fraction #4) was treated separately with both enzymes. Since upon enzyme inactivation with acid, the supernatant was slightly greenish and chlorophyl has an absorption peak at 260 nm, we determined DNA and RNA concentration directly in the Pellet (i.e. protein concentrate or fraction #6) of the control and enzyme treated samples.

RESULTS & DISCUSSION

Cell wall consists mainly of complex polysaccarides and murein, the typical structural macromolecules of bacterial cell walls (Soeder, 1978) and if left intact, is an obstacle to digestability of cell components. To remove the cell wall it is possible to use chemical methods which include the use of urea, guanidine, sodium hydroxide (Mitsuda et al., 1969, Huang and Rha, 1971), alkaline biocarbonate buffer (Tannenbaum et al., 1966), acetic acid, oxalic acid, citric acid (Samerjima et al., 1971) methanolic hydrogen chloride (Tamura et al., 1972) and ethanol acetone (Lee et al., 1979). It is also possible to use enzymatic methods which include incubation of active cells at the optimum temperature to induce the lytic reaction of endogenous enzymes or addition of enzymes to lyze the cell wall (Hedenskog et al., 1969; Maul et al., 1970; Castro et al., 1971, Carenberg and Heden, 1970).

Although each of above methods has its own advantages, we, however, due to the potential limitations and restraints of CELSS, focused on simple physical methods for cell wall disruption. The physical methods generally involve the rupture of cells with a high-pressure press, freeze-thaw treatments, and sonication, as well as high-speed ball mill grinding, high pressure homogenization, and high-speed mixing (Hedenskog and Mogren, 1973; Lee et al., 1979; Cunningham et al., 1975; Dunnill and Lilly, 1975).

Comparison of physical cell wall disruption methods by direct Scanning Electron Microscopy (SEM) showed that, under our experimental conditions, homogenization with glass beads results in more disruption than the other methods. Consequently, homogenization was chosen as our method for breakage of algae cell walls. Fig. 4 shows the comparison of the microscopic inspection of intact algae cells with homogenized (30 min) cells. Upon completion of above stage and removal of beads with sieve #40 (420 µm openings), we obtained a homogeneous mixture of cell wall fragments, various released cytoplasmic proteins and cell organelles, nucleic acids, and pigments, from which we were interested in separation, concentration, and isolation of proteins. The utilization of the remaining components could be the subject of additional research activities in the area of by-product management.

Major nutritional components of algae flour (freeze dried broken cells) are shown in Table 1. Our values are in good agreement with those reported in the literature (Becker, 1981; Jurkovic et al., 1983; Piorreck et al., 1984) indicating that continuous algal culture results in algal biomass similar to batch-type products.

Fig. 5 shows the stepwise procedure for preparation of various algal fractions. Adjustment of broken cell suspension to pH 10.0 was based on the known pH-solubility curves for plant proteins (Wolf, 1978) and according to the methods of Hedenskog and Mogron (1973) and Hedenskog (1978). At this pH, proteins and associated molecules are soluble and therefore, upon centrifugation, undesirable cell walls (containing murein and disturbing carbohydrates), along with some fragments and organelles are excluded from the product. This results in algal crude protein (Fraction #4) which theoretically have higher digestibility values.

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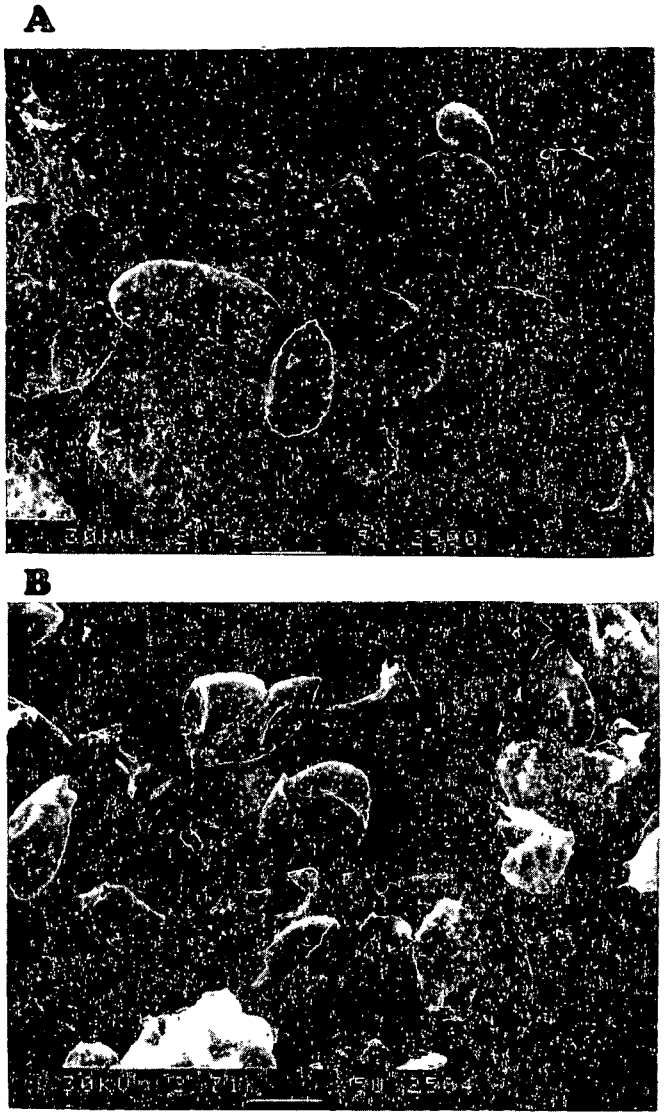

Fig 4) Scanning Electron Micrograph of Green algae (Scenedesmus obliquus) cells (A) and homogenized cells for 30 min (B).

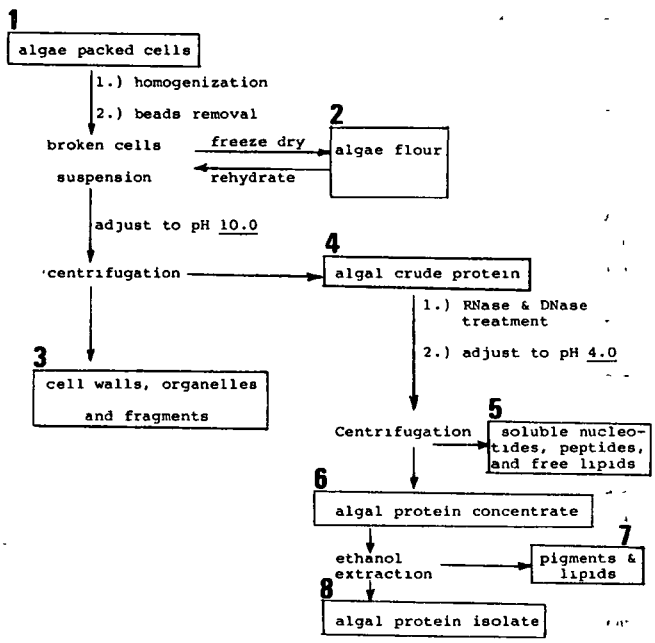

Fig 5) Stepwise procedure for preparation of various algae fractions

At this stage, attempts were made to remove another undesirable component of the algal system, i.e. nucleic acids. Nucleic acids, insoluble below pH 4.5, if ingested directly, lead to the elevation of blood uric acid levels as a result of their degradation to the purine bases, adenine and guanine, and the *in vivo* oxidation of these latter. Uric acid with extremely low water solubility cannot be further degraded and is only partially excreted. This leads to gout, diseases affecting the joints and to the formation of "stones" in, for example, the kidneys and the bladder (Hudson, 1980). Reduction of the levels of nucleic acids is thus of special importance.

The generally accepted safe level of nucleic acid intake in the human is 2 grams per day (Scrimshaw, 1975), and since the total concentration of nucleic acids (RNA & DNA) of the studied algae is found to be 6.0%, this would limit, the algal consumption to about 33 grams per day. Thus, if algal proteins are to be used as a major ingredient in foods in space habitats, reduction of nucleic acids is an essential step.

There are numerous methods for reduction of nucleic acid content in cell suspensions and in cell homogenate. These methods have been reviewed by Sinskey and Tannenbaum (1975), Litchfield (1977), Chen and Peppler (1978), Hedenskog (1978) and Gierhart and Potter (1978). Cell suspensions have been treated with acid (Peppler, 1970), aqueous ammonia (Ayukawa *et al.*, 1971; Akin and Chao, 1973), heat shock/pancreatic RNase (Castro *et al.*, 1971), heat shock/Na_2HPO_4 solution (Canepa *et al.*, 1972), MeOH/HCl mixtures (Tamura *et al.*, 1972), heat shock (Tannenbaum, 1973), EtOH/HCl mixture (Akin and Chao, 1974) heat shock/pH 5.0-5.5 (Akin, 1974), heat shock/carboxylic anion (Sinskey and Tannenbaum, 1975), and NaOH or aqueous ammonia (Viikari and Linko, 1977).

Reduction of nucleic acids in cell homogenates have been reported with NaCl 3%/50°C - pH 5.6 (Lindblom and Morgan, 1974), pH 6.0/80°C (Vannanuvat and Kinsella, 1975), high temperature-low alkali, or low temperature-high alkali (Newell *et al.*, 1975), 100°C/pH 6-8 (Robbins, 1976), extracellular RNase (Fazakerley, 1976), and succinylation after cell disruption (Shetty and Kinsella, 1979).

Enzymatic treatment results in depolymerization of nucleic acids into mono and oligo nucleotides and since the latter are soluble at lower pH's, acidification of the algal crude protein (to pH 4.0) will result in exclusion of undesirable nucleotides (along with soluble peptides and free lipids) from protein concentrate into supernatant.

Treatment of pure RNA with RNase showed that optimum condition at 37°C for maximum absorption (260 nm) are: pH 5.0, Enzyme/substrate ratio of 1/200 (w/w) and reaction time of 8 min. Similarly, the optimum conditions for DNase at 25°C are: pH 5.0, Enzyme/substrate ratio of 1/1 (w/w) and reaction time of 10 min.

When the algal crude protein (#4) was treated under above optimum conditions, results showed 96.5% decrease in RNA concentration and 79% decrease in DNA concentration.

Table 2A shows the comparison of RNA and DNA concentrations in various algae fractions before and after enzymatic treatment. Reduction of total nucleic acids concentration of algae protein concentrate (#6) to 0.81% increases the safe consumption level of this protein from approximately 20 g (non-treated) to approximately 250 g per day. Similarly reduction of total nucleic acid concentration of protein isolate (#8) to 1.04% increases the safe consumption level of this protein from 15 g to 190 g per day (Table 2B).

Extraction of high concentration of pigments (mostly chlorophyll a) and lipids from the algae protein concentrate (fraction #6) results in removal of almost all green color and consequently the algae protein isolate (fraction #8) has a "light olive color" upon freeze drying. Removal of the pigments and lipids enhances the acceptability of algal products from the color and flavor points of view, but causes denaturation of algae proteins (regardless of ethanol extraction temperature) and therefore water insolubility of the final product. This side effect may result in loss of some useful functional properties during the following fabrication processes. Yield (%), protein concentration and protein recovery (%) of various algae fractions is represented in Table 3. Although the total recovery and protein concentration of protein isolate seem rather reasonable, attempts should be made to increase these values.

Above results show that green algae can be processed and upgraded (with a reasonable yield) to algal protein isolate by removing 3 major undesirable cell components (cell wall, nucleic acids, and pigments and lipids). This will enhance the potential nutritional and organoleptic acceptability of algae products while some functional properties may be lost and need to be restored or avoided. Further research on optimization of each processing step and consequently improving the yield and quality of final product (upon protein characterization) is needed before any study on nutritional, toxicological, and technological aspects is planned. The latter studies are of prime importance and should be carried out before the actual utilization of algae as a protein source in space habitat.

Table 1) Nutritional composition of algae flour (freeze dried) broken cells). Solid content of whole algal cell prior to freeze drying was found to be 23%.

Proteins	52.6%
Nucleic Acids:	
RNA	4.3%
DNA	1.7%
Pigments & Lipids	15.0%
Carbohydrates	16.5-18.5%
Ash	7-8%
Moisture Content	1-2%
Total	98.1-102.1%

Table 2A) Concentration of RNA and DNA in various algal fractions before and after enzymatic (RNase & DNase) treatment.

fraction #	% RNA		% DNA		total N.A. reduction upon Enz. treatment (%)
	before	after	before	after	
2 (algae flour)	4.3	-	1.7	-	-
4 (algal crude protein)	6.8	-	2.0	-	-
6 (algal protein concentrate)	7.4	0.26	2.6	0.55	92
8 (algal protein isolate)	9.9	(0.35)	3.3	(0.69)	92

Table 2B) Amount of algae (grams) containing safe consumption level of nucleic acids i.e., 2g/day (Scrimshaw, 1975).

fraction #	Before Enz. treatment	After Enz. treatment
2(algae flour)	33	-
6(algal Protein Concentrate)	20	247
8(algal Protein isolate)	15	190

Table 3) Yield (%), protein concentration (%) and Protein recovery (%) of various algae fractions without enzymatic treatment.

fraction #	yield(%)	Protein concentration (%)	Protein Recovery %
2 (algae flour)	100	52.6	100
4 (algal crude protein	65	57.6	71.2
6 (algal protein concentrate)	51.6	53.4	52.4
8 (algal protein isolate)	36.6	70.5	49.1

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BLSS, A European Approach to CELSS

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ABSTRACT

Controlled ecological life support systems (CELSS) have for some years been subject to intensified studies and experiments in the U.S. and the U.S.S.R., and in Europe and Japan as well in recent years.

The presently planned Space Station concepts foresee an early implementation of water and oxygen recovery in order to reduce resupply weight and volume. In view of expected increase in station and crew size the spacecraft payload limitations will require that the carbon, or food, recycling loop, the third and final part in the life support system, be closed to further reduce logistics cost. This will be practical only if advanced life support systems can be developed in which metabolic waste products are regenerated and food is produced.

Dornier System has in recent years undertaken an effort to define requirements and concepts and to analyse the feasibility of a Biological Life Support System (BLSS) for space applications. Analyses of the BLSS energy-mass relation have been performed, and the possibilities to influence it to achieve advantages for the BLSS (compared with physico-chemical systems) have been determined. The major problem areas which need immediate attention have been defined, and a programme for the development of BLSS has been prosed.

A feasibility study of a closed life support system for plant and animal experiments in space has been initiated and results will be verified by bread-board testing of selected alternatives. The principle is to form a chain of ECO-groups consisting of food producers, consumers and decomposers, of which one (plants or animals) will contain the life science test species. Considered possibilities are combinations of aquarium concepts, algae reactors and vertebrate vivaria.

This paper discusses the BLSS feasibility analyses activities performed in Europe, the ongoing experimental/development work and future planning for European BLSS activities.

FOR EXTENDED DURATION MISSIONS in space the practical supply of basic life-supporting ingredients represents a formidable logistics problem. The weight at launch and the storage volume in weithlessness of water, oxygen and food in a conventional non-regenerable life support system are directly proportional to the crew size and the length of the space mission. In view of spacecraft payload limitations, the inescapable conclusion is that extended-duration manned space missions will be practical only if advanced life support systems can be developed in which metabolic waste products are regenerated and food is produced.

Only a Biological Life Support System (BLSS) *, which not only satisfies the space station environmental control function requirements, bus also closes the food cycle, can meet all the expected requirements. A BLSS must be a balanced ecological system, biotechnical in nature and consisting of some combination of human beings, animals, plants and microorganisms integrated with mechanical and physico-chemical hardware.

* Biological Life Support System (BLSS) is synonymous to Controlled Ecological Life Support System (CELSS) in this paper.

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Numerous scientific space experiments have been delineated in recent years, the results of which are applicable to the BLSS concept. To ensure that the efforts expended by various international bodies aim toward a common goal, the coordination with existing Spacelab and Shuttle utilization programmes is of major importance to avoid duplication of effort and to gain access to valuable data as early as possible. The analysis reported here is a result of a cooperative effort undertaken by Dornier System and Hamilton Standard in recent years to define requirements and concepts, and to analyze the feasibility of BLSS for space applications. The development of BLSS relevant experiments has also been initiated in Europe.

STATE OF THE ART

The development of manned space activities will most likely continue along the evolutionary lines that have so successfully guided the space programme to date. Along with progressively growing crew sizes, mission duration and complexity have increased dramatically since the first orbital flights in 1961 - 1962. Mission duration has progressed from the one to three orbits of the first Vostok and Mercury flights to the 84 days of the third Skylab flight and the 211 days of Salyut. From the initial, single objective of survival, mission objectives have increased to the achievement of major experiments, and the accomplishment of major operational missions, such as satellite launch, deployment, capture, repair and redeployment.

The Space Transportation System (STS), Shuttle Orbiter and Spacelab, are opening up the future expansion of manned space activities. The baseline STS capability is a seven day on-orbit mission.

Future use of space stations and larger scale operations are forecasted to continue in a progressive manner [1]*. In concert with the evolution of man's activities in space, the technology to support these activities will require progressive development of today's space systems. Of major importance is the life support system. The latest U.S. and European manned space vehicles, the Space Shuttle Orbiter and the Spacelab, contain the same life support systems with expendable supplies, such as the systems used on the earlier manned space flights. However, the next phases of manned space flight development will provide substantial impetus to improve life support technology, and to reduce the dependency upon these expendable technologies. Figure 1 shows how improvements in life support technology might be implemented in conjunction with the mission growth scenario.

* Numbers in parenthesis designate references at end of paper.

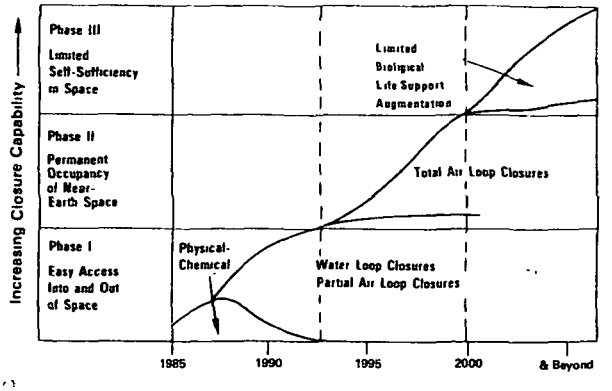

Fig. 1 - Prospective evolution of life support systems [1]

The next U.S. and European manned space objective is a Space Station. This permanently manned facility will be resupplied on 90-day intervals and have a crew size of 6 - 8 astronauts. Such an in-orbit system is envisioned to have a large role in the commercialization of space activities, as well as playing a key role in continued development of space technology, primarily in the area of in-orbit operations. Because resupply from Earth of metabolic expendables (O_2, clean H_2O, food) incurs a high launch cost the Space Station life support system is expected to regenerate water and oxygen.

Beyond the initial Space Station, future manned space missions include various missions that require large teams of humans working and living in space for extensive periods of time in permanently-inhabited large space stations. These space habitats will require the carbon loop to be closed to further reduce logistics costs. This recycling of carbon will only be practical if advanced life support systems can be developed in which metabolic waste products can be used to produce food (Figure 2).

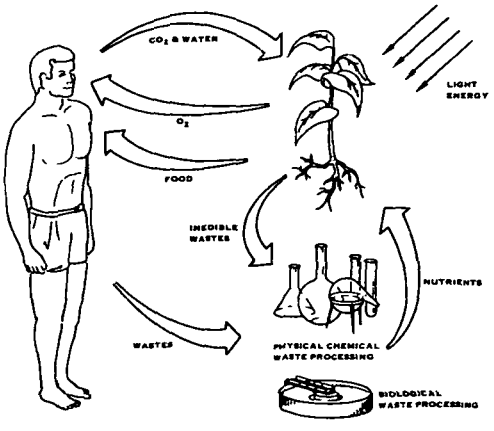

Fig. 2 - Principal Biological Life Support System (BLSS)

Initial efforts to investigate advanced life support systems of the ecological/biological type to close the carbon loop (food supply), Figure 3, have been undertaken in the U.S. (Controlled Ecological Life Support Systems, CELSS) and in Europe (Biological Life Support Systems, BLSS) in recent years. During this decade, continuing efforts will concentrate on feasibility studies, investigations of specific development issues, and flight experiments to prove the viability of selected detailed designs or to provide basic scientific information in preparation for large scale testing on board a space station in the 1990's. A indicated in the literature, intensive experimental studies concerning BLSS are also being conducted in the U.S.S.R. and Japan as well. Both terrestrial and space experiments are being planned or performed.

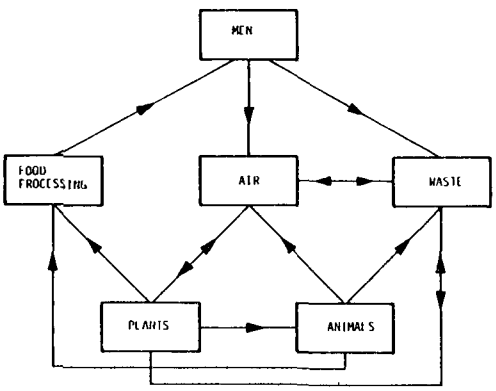

Fig. 3 - Principle carbon mass flow in a closed system (BLSS)

The benefit of BLSS is primarily an economic one, because the cost of launching supplies into orbit to support manned space activities can be reduced by the use of a BLSS. The first, and relatively near potential application for BLSS is on a space station in a low earth orbit (LEO). An estimated systems trade-off between a non-biological (physico-chemical) regenerative system and a biological system with ∿80% food closure is given in Figure 4.

Depending on the mission type and crew size the pay off varies from 6 - 7 years for a 4-man crew to about 1 1/2 year for a 100-man crew in LEO.

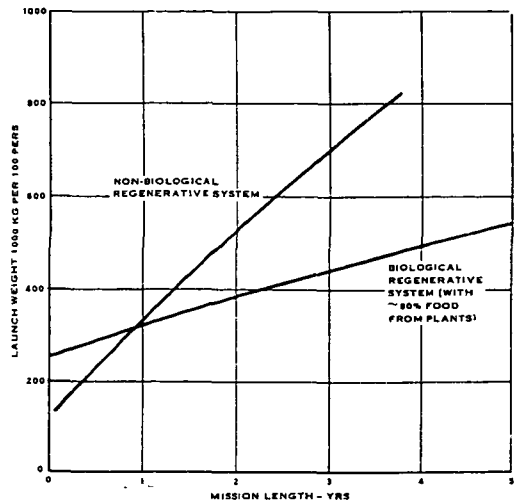

Fig. 4 - Estimated system trade-off for life support system alternatives

BLSS REQUIREMENTS

In defining BLSS characteristics, it is important to consider potential space applications, which dictate BLSS functional requirements. A permanently manned space station or base has been used as the model for the following BLSS discussions, because this application embodies the essential complexities of most BLSS uses. As BLSS will represent only one of many subsystems integrated to form the space station, the BLSS design must take into account all potential inputs (e.g., gases, chemicals) from other subsystems if the resulting space station ecology is to be balanced and stable.

Space station life support functions can be more definitively specified as:

- Oxygen Production
- Carbon Dioxide Control and Reduction
- Contaminant Gas Control
- Two Gas Control and Pressure Regulation
- Humidity Control
- Thermal Control
- Solid Waste Reclamation
- Waste Water Reclamation
- Radiation Protection
- Illumination
- Artificial Gravity
- Food Supply (production and supply).

Ultimately, BLSS functional requirements for space application will be to supply oxygen, water and food for support of human life on a continuous basis, while maintaining a balanced, stable spacecraft ecology. The BLSS must satisfy both the Environmental Control and Food Production functional requirements of the space

station listed above. While the precise BLSS components will be highly dependent on the space mission, it will probably consist of humans, animals, plants and microorganisms integrated with other supporting physicochemical components.

In an ideal scenario, a BLSS would be capable of perfect:

- metabolic balance between man's oxidative process and plants regenerative process,
- waste water reclamation, and
- mass-balanced regenerative food/waste cycle.

The closed system as presented in Figure 5 would represent this case. In a closed system, where the food supply might include both animal and plant species, no unusable residues would be produced. That is, a perfect regerative balance of input and output quantities from human, animal and plant species would be maintained. In practice, however, total BLSS closure will not be achievable. At best, BLSS closure will be approached incrementally and only after intensive biological research effort.

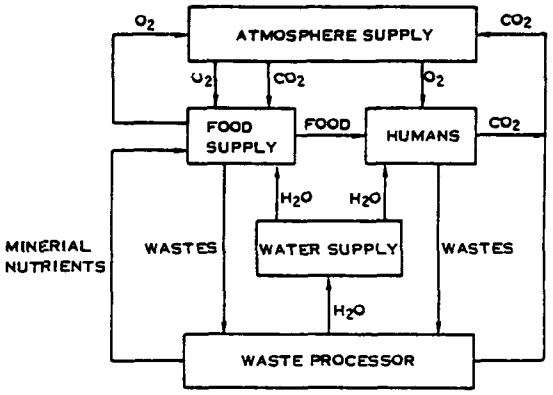

Fig. 5 - Closed BLSS

To expand upon the concepts introduced above, the BLSS must be balanced in the sense that proper proportions of CO_2, O_2, biomass, water, food reserves, etc., are maintained. The precise nature of this balance relates directly to BLSS's regenerative ability to convert waste products to usable products. In any practical BLSS, supplement additives to the system will periodically be required to maintain the desired ecological balance, because some unusable waste residues will always be produced. Such BLSS systems are said to be partially closed, Figure 6.

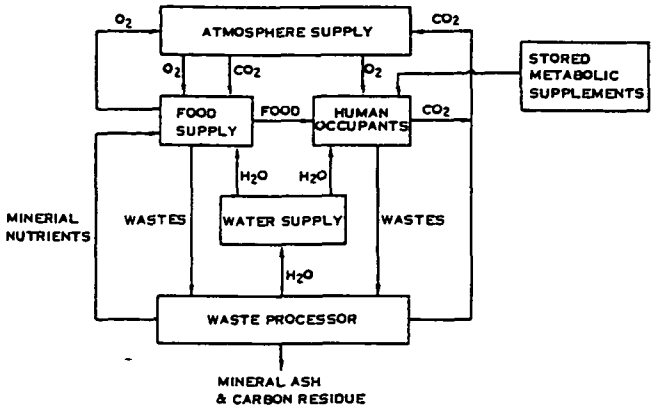

Fig. 6 - Partially closed BLSS

Assessing the required life support functions (oxygen supply, food production and water reclamation) for a BLSS indicates that the food production requirement is the design driver for higher plants. A system sized for food production will be in the position to handle the other life support functions without an increase in size. Analyses of the BLSS energy-mass relation have been performed, and it appears possible to achieve advantages using the BLSS compared to physico-chemical systems. At equal energy consumption for a BLSS and a physico-chemical system, the break-even point of mass is in the order of 7 years. If the phototrophic efficiency could be increased over the 2% used in this analysis the energy consumption would be higher for the BLSS, but it would show a weight advantage for shorter mission durations [2].

BLSS DEVELOPMENT APPROACH

The development of an operational biological life support system for space requires dual development paths [2]. In parallel to the selection of species plants and animals, the improvement of culturing methods and of waste treatment by experimental investigations, and mathematical models will be needed to decrease development risks of the prototype BLSS.

The development process (Figure 7) starts with the specification of the human diet and the vitamin and trace mineral requirements. Compatible with these human requirements and the environmental conditions of a space station, the next step would be to select the plant and animal species required. This selection will be reevaluated and retested as the development of a BLSS makes progress in the following areas:

- higher yield of cultures,
- waste treatment, and
- control mechanisms.

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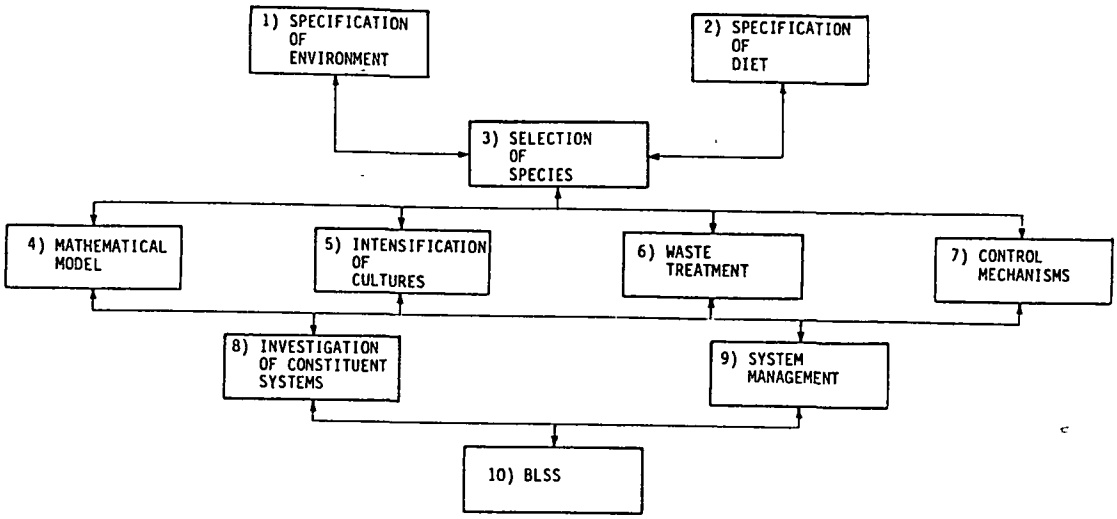

Fig. 7 - Idea of the development process of a BLSS

Many single experimental investigations in various disciplines will be necessary for the evaluation of the biological, chemical and technical basis for these areas before they can be integrated into subsystems, whose functional coupling and reliability under working conditions can be tested.

The theoretical approach, going hand in hand with the experimental one, will use mathematical models. These mathematical models should describe the functional couplings between all system components as well as their dynamic behaviour. The models should also define system stability and eventually form the basis for computerized control and management of the system, including problem prediction, trend analysis, crop forecasting, and logistical requirements predictions.

The early state of development of the BLSS system is reflected by the large number of issues yet to be resolved in the definition of an operational system. Table 1 summarizes some basic developments yet to be undertaken in the areas of environment control, agriculture, aquaculture, food synthesis and processing, diets, and waste conversion. A development programme as outlined in Figure 7 is envisioned to sequentially address these issues in the development of a BLSS system [2 & 3].

Within the large list of BLSS issues to be resolved, there are a number of early technology tasks that can be performed in an initial test and development programme to lay a technological foundation for the eventual BLSS system evolution. These early key tasks are listed in Table 2.

These problems have to be subdivided into ones that absolutely require studies in space, and ones that can be studied and solved in terrestrial research programmes. Furthermore, priorities should be set as to whether the problem is relevant in the very near future (short-term relevance, pre-pilot type) or not (long-term relevance, pilot type).

Table 1 - Basic BLSS Development Issues

REQUIRED BLSS SCIENTIFIC AND TECHNOLOGY DEVELOPMENTS
Environment
- Materials selection - Atmosphere selection - Gravity selection - Radiation shielding requirements and methodology - Ecosystem tradeoff studies - Chemical analysis and control of contaminants and toxicants - Illumination requirements - Solar reflectors and filters
Management and Control
- Critical biological performance parameters - Biological sensor development - Definition of biological stability criteria - BLSS mathematical models - BLSS management and control philosophy
Agriculture
- Plant culture and physiology in space environments - Concepts to reduce spatial requirements - Equipment concepts for cultivation and harvesting - Radiation effect on genetic drift germination - Plant growth without soil - Forced growth effects on plants - Plant cycle photosynthesis efficiency - Plant hormone activity in micro-gravity - Plant production of toxic gases
Aquaculture
- Food-producing ecologies based on waste conversion - High yield, high nutrition plant production and harvesting - Photosynthesis process
Food Synthesis
- Acceptable microbiological sources and production methodology - Acceptable chemical synthetic production of protein and carbohydrates
Food Processing
- New concepts for food preparation processing, storage, and distribution to reduce equipment and resource requirements - Improved food preservation and packaging methods
Diet Planning
- Human nutritional requirements - Food and food-source selection criteria - Nutritional equivalency of various food sources - Physiological and psychological acceptability aspects of nonconventional diets and food sources - Definition of crop/plant scenarios - Digestive tract adaptability
Waste Conversion and Resource Recovery
- Physico-chemical processes, particularly mineral separation and recovery - Microbiological processes - Regenerative chemical filters - Chemical separation methods - Auxiliary non-food products from wastes (e g , paper and tools) - Plant waste byproduct processing.

Table 2 - Problems to be Studied in Early BLSS Development

TASK	Pre-Pilot Type		Pilot Type	
	Terrestrial	Space	Terrestrial	Space
O-g influence during cultivation	x	x		x
O-g influence on culture-methods	x	x		x
Solar radiation in PAR region impact on biological material	x		x	x
Cosmic radiation	x	x		
Optimization of biological material	x	(x)	x	
Optimization of cultivation methods	x	(x)	x	
Optimization of harvesting methods	(x)	(x)	x	x
Energy recycling	x		x	
Waste recycling	x		x	
Monitoring and Control	x	x	x	x
Improvement of mathematical modelling	x		x	
Selection of diet	x		x	
Development of large area windows for PAR and IR	x	x	x	x
Refined theoretical model	x		x	

( ) = need for exp. still to be defined
PAR = Photosynthetic Active Region
IR = Infrared

Generally speaking, only those problems need to be studied in space, which:

(i) require a micro-gravity environment, and/or

(ii) are cosmic radiation dependent.

As to i), perhaps problems arising in the micro-gravity environment of a BLSS may be solved on earth by studying the problems under increased g-force levels and directional attitudes of gravity, and then extrapolating the results to O-g. This approach, in connection with sophisticated mathematical modelling, might be successful. If experiments have to be conducted under micro-gravity, it seems possible that only verification experiments may be necessary.

As to ii), it is clear that the simulation of cosmic radiation on earth is very difficult, and that appropriate experiments may have to be performed in space. However, the composition of cosmic radiation and its distribution in space is relatively well known, so that first order approximations are possible for certain experiments.

For all experimental activities, a prerequisite is that they focus on the applicability of certain biological features for BLSS. Therefore, questions concerning problems of basic life science are not to be studied, but results of such experiments might provide answers to certain questions relevant to BLSS.

PRE-PILOT STUDIES - should center around the problem of providing the crew with a certain amount of fresh greens. The culture methods are characterized by the use of prepared beds or pots which contain a medium either in the form of solid fertile 'soil' (agar plate) or spongelike substances. The interface of the BLSS with the spacecraft and with outer space (sunlight) should be as simple as possible. Direct sunlight would be preferred from an energy point-of-view, but because of multiple light-dark periods during each 24-hour day in low earth orbit, solar powered artificial light may be required.

The harvesting process should take place by cutting plants during their vegetative period. Species able to perform vegetative reproduction should be selected to shorten the duration between the harvesting periods; the generative period during growth should be by-passed. Vegetative reproduction is usually supported by the method of stem-cutting. This method is also less crew-time consuming than sprouting from seed.

PILOT STUDIES - focus on the design and testing of a terrestiral reference system which simulates the life support system with its biological subsystems intended for flight application. Reference systems have in the past been designed and tested along with the development of physico-chemical subsystems.

Whereas in pre-pilot studies principle aspects of BLSS are experimentally investigated, the aims of pilot design and testing of a reference system are to verify the selected principles for the closure of the water, atmosphere and carbon loops as a system. The successful experimental work performed to date with such systems led to the conclusion that the concept of a reference system is valid. Pilot studies should include both terrestrial and space activities.

It is only in the final stage of the development of BLSS that pilot studies will occur in space. At this stage of development, complete biological subsystems are flown, possibly as some kind of parallel system to physico-chemical subsystems, activated only during a certain phase of the mission. Such a mission will occur before complete BLSS are implemented as the main life support system.

DEVELOPMENT OF BLSS EXPERIMENTS

The BLSS studies have indicated two blocks (pre-pilot and pilot type) of experiments and analysis which are required for the support and promotion of the development of BLSS (Table 2). The development of specific flight experiments should follow the generalized flow diagram in Figure 8. This approach takes into account the known typical BLSS design parameters for different types of species, and can also be used for

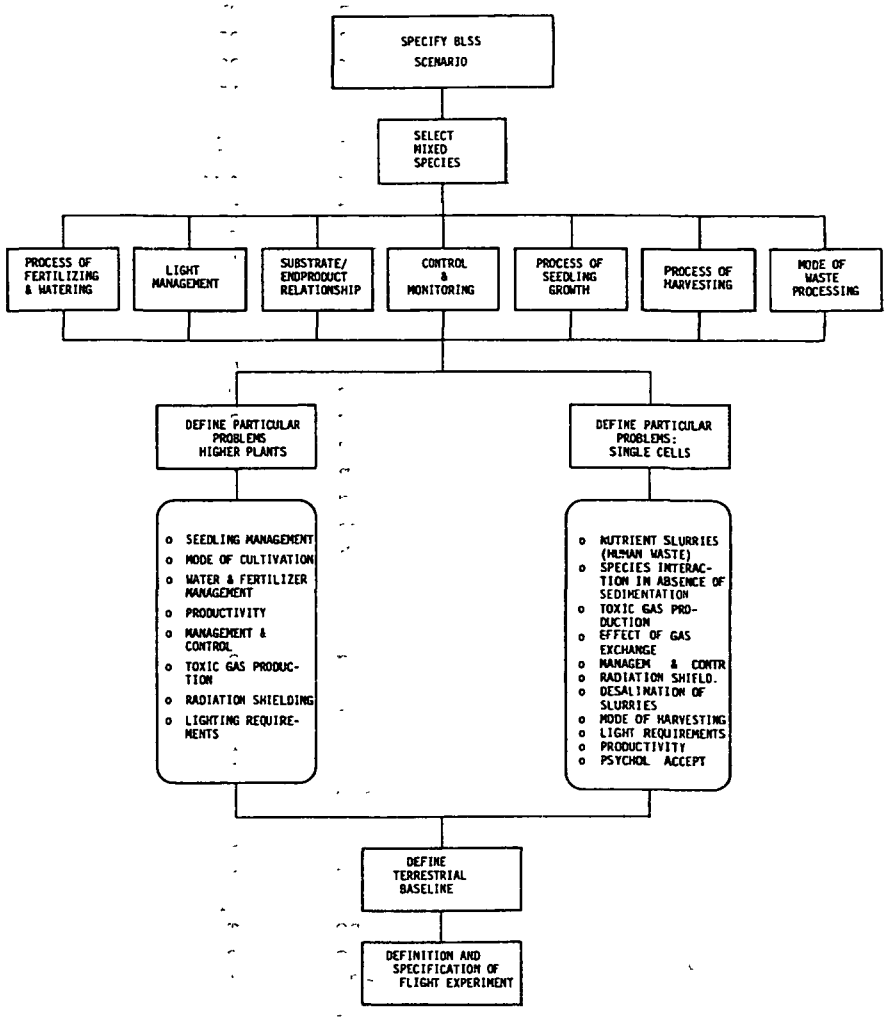

Fig. 8 - Development of flight experiments for BLSS

the definition of new BLSS flight experiments and to evaluate modifications to planned expements. A preliminary programme has been proposed indicating some potential BLSS experiments. These experiments investigate those areas with immediate impact upon the successful integration of a regenerable life support system into future manned space activities.

Tasks of immdiate importance from a life support system development point-of-view are:

- investigations concerning micro-gravity,
- investigations concerning cosmic radiation,
- development of large area windows for radiation in the PAR-region,
- investigations concerning harvesting and cultivation in micro-gravity,
- monitoring, control and sensor technology, and
- waste processing.

Cosmic radiation studies are already planned, but those experiments dealing with microgravity and PAR-windows are only partly defined. Any efforts related to the PAR-windows should include systems analysis studies in the areas of:

- the correct wavelength needed for optimum growing conditions,
- avoidance of excessive heat load into the spacecraft, and
- use of day/night growing cycles.

Concerning the cosmic radiation investigations, advanced experiments are planned and, in this case, the interpretation of results, and the subsequent influence on species selection are the major tasks in the BLSS development.

New experiments should have the dual goal of advancing the basic scientific research while meeting the BLSS requirements.

Presently two projects are being performed in Europe with a direct link to BLSS:

- Solar Plant Growth Faciltity (SPGE) and
- Environmental Life Support System Technology Study (ELSS).

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SOLAR PLANT GROWTH FACILITY - The Solar Plant Growth Facility is to be designed as a reusable life science facility rendering possible investigations with respect to future biological life support systems (BLSS).

By conducting long term experiments (about 6 months), the results should be useful to enhance the technological background needed for the development of BLSS; for this purpose a certain biological sample is exposed to well defined environmental conditions in a low earth orbit and factors which are expected to influence the design of BLSS (metabolism, survival rates, morphogenesis,..) are studied. By this way, one will gain experience in handling and cultivating larger amounts of biological material necessary for providing food and a suitable atmosphere in future manned space missions.

The data collected inflight shall establish information on:

- Metabolism of the plants, that is
  - O_2-production and consumption
  - CO_2-consumption and production
  - H_2O-transpiration,
- Morphogensis of the plants,
- Development of flowers and seeds,
- Regeneration after cutting, vegetative reproduction,
- Light input to the plants
  - total amount
  - cycle (60 min. day, 35 min. night), and
- production of gaseous trace contaminants (e.g.C_2H_4).

The principle schematic of the SPGF is given in Figure 9. The overall dimensions are 660 x 1360 x 900 mm and the overall weight 152 kg. Technically the SPGF will be used to verify:

- the illumination concept (window and shutter),
- the atmosphere regeneration and gas supply (absorption of CO_2, absorption of O_2 and supply of CO_2),
- observation methods,
- proposed stem-cutting concept,
- proposed pollination concept,
- watering and nutrient supply concept, and
- thermal humidity and dehydration schemes.

A breadboard unit of the SPGF is shown in Figure 10.

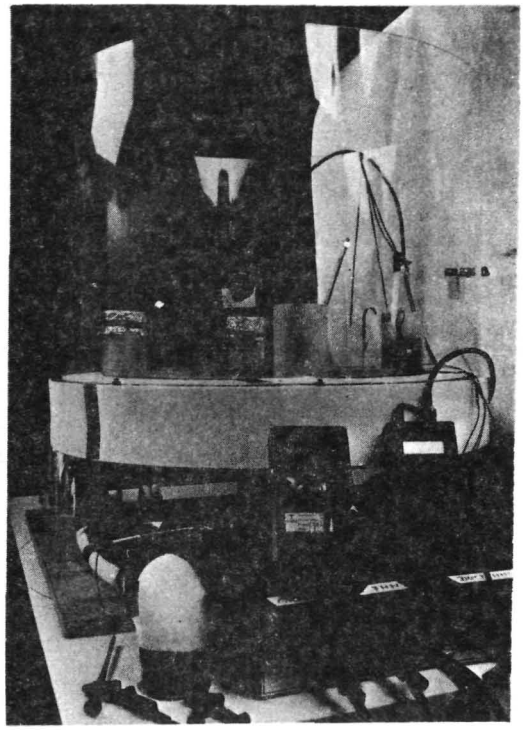

Fig. 10 - SPGF Breadboard Model

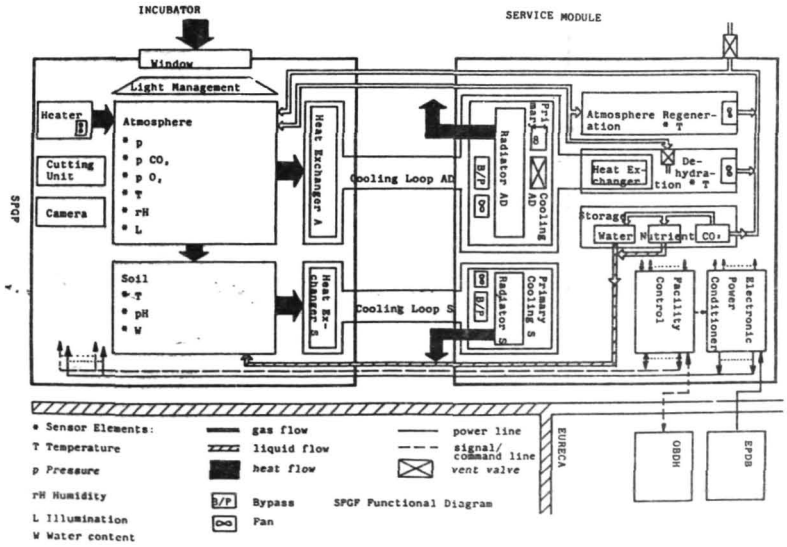

Fig. 9 - SPGF Schematic (Source: ORS)

ACKNOWLEDGEMENT

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CELSS Experiment Model and Design Concept of Gas Recycle System

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ABSTRACT

In order to prolong the duration of manned missions around the earth and to expand the human existing region from the earth to other planets such as a Lunar Base or a manned Mars flight mission, the CELSS becomes an essential factor of the future technology to be developed through utilization of Space Station.

The preliminary SE&I (System Engineering and Integration) efforts regarding CELSS have been carried out by the Japanese CELSS concept study group for clarifying the feasibility of hardware development for Space Station Experiments and for getting the time phased mission sets after Fy 1992. The results of these studies are breifly summarized and thereafter, the design and utilization methods of a Gas Recycle System for CELSS experiments are discussed.

1. PROPOSED EXPERIMENTS AND ITS MISSION ANALYSIS

According to NASA's call for international participation in the Space Station program, data sources for Space Station Utilization concepts in many fields have been collected at the Japanese Space Station Symposium held in October 1982. Among the papers presented at this Symposium, eleven experiment proposals as shown in Table 1 related to CELSS have been extracted as the data source for CELSS mission analysis. Using these data sources, extensive study for clarifying the development feasibility of hardware necessary to conduct the CELSS experiments with state of art equipment have been conducted.

Each proposed theme was divided into the research items closely connected with experiment hardware and the necessary time spans for developing hardware were investigated considering the technological maturity and effectiveness for experiments. Table 2 shows the strawman CELSS experiment concept and is described in next section.

2. CELSS EXPERIMENT CONCEPT

The CELSS is the technology for making a stable ecology among animals and plants without re-supply of materials and using a special controlled environment. Therefore the CELSS would be divided into two sections; the environmental control section and the cultivating and breeding section of plants and animals. In the environment control section, at least three major systems, shown Fig. 1, should be installed for sustaining the gas environment, for water recycle and for decomposing waste materials into a fertilizer solution.

In the cultivating and breeding section, the Algae and higher plant cultivation systems for converting carbon dioxide to oxygen and for producing food are to be installed. In addition aminal and fish breeding systems for obtaining animal protein should also be installed.

In order to develop CELSS technology, it is necessary to take a long time span as described in previous section because basic ground based experiments related to the CELSS are required before developing the flight experiment hardware, and also because the data of stabilities about the morphogenesis and physiology of the higher plants and algae in space environment have not been fully accumulated at the present time.

Based on the above considerations, the time phased mission sets for utilizing the space station were determined as a Japanese strawman CELSS mission model. (Reference 1) (Nitta, 1984)

According to this mission model, the first time mission is to be conducted during 1992 - 1995 for evaluating the higher plant and algae cultivation methods and for summing up the available data about the stability of

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Table 1 Proposed Theme

No.	Theme	Description of Content
EL-01	Agricultural Laboratory in Space	Fungi production for cellulose decomposition, animal and fish breeding for food production, higher plant and algae plantation for food production and gas conversion, salt accumulation plant for sodium chloride extraction, hydrophyte planting for nitrogen fixation, methanogen fermentation for waste management and construct the closed ecology with bio species mentioned above.
EL-02	Space Agriculture Experiment	Vegetable planting for food production and gas conversion. Solar light supply system for photosynthetic reaction of vegetable.
EL-03	Study on Space Agriculture and Closed Ecological Life Support System	Gas recycle system for stabilizing gas environment, water recycle system for suppling the necessary water, wet oxidation system for waste management, higher plant and algae cultivator for food production and gas conversion, physico-chemical sodium extraction system, and low gravity generator for testing geotropism.
EL-04	Non-Gravity Plant Experiment System	Chetotaxis experiment in 0G. Organella growth experiment in 0G and geotropism experiment of higher plant.
EL-05	Project for establishments of Breeding and Management System of several Higher Animals under Space Environment	Reproduction, growth, enbryogenesis and genetics experiment in 0G using Mouse, Quail and Tilapia, and breeding technology development for future food production.
EL-06	Micro-Ecological System in Space Station	Gas recycle system, water recycle system and incineration waste management system are recommended.
EL-07	Microbial Fermentor in very low gravitational force	Space-use fermentor design for practical use of microbes in environment control.
EL-08	Field of Plant Research in Space Station	Cell culture, pathogen study and protein rich flour separation using 0G.
EL-09	Plant Experiment Subsystem	Plant cultivator, phytotron design.
EL-10	Biochemical Studies on elementary cycles in a Closed Ecosystem	Simple ecosystem and immobilized enzyme bio-reactor for supplementary food production.
EL-11	Space Station with an Artificial Gravity	Large scale ecological life support experiment station.

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Table 2 Proposed Research Items

Exp. No.	Items contained	1991 ~1994	1995 ~1998	1999 ~	Reasons
EL-01	(1) Higher plant for food	V	V	V	Essentially required for CELSS
	(2) Microbial waste management			V	After the sludge problem is solved
	(3) Sodium accum. plant	V	V		Easy to conduct with phytotron
	(4) Hydrophyte for fertilizer			V	After the effectiveness is examined
	(5) Fish and Animal		V	V	Essential for animal protein
	(6) Fungus			V	After the effectiveness is examined
	(7) Integrated ecological test		V		Possible if physico-chemical systems introduced
EL-02	(1) Experiment module		V		Dedicated mission
	(2) Solar collector system	V	V		Required for higher plant
	(3) Vegetable for foods	V	V		Same as EL-01 (1)
EL-03	(1) Experiment module		V		Same as EL-02 (1)
	(2) Animal and Fish		V	V	Same as EL-01 (5)
	(3) Gas separation and reservoir		V	V	Essential for eco-stabilization
	(4) Higher plant for foods	V	V	V	Same as EL-01 (1)
	(5) Algae for gas exchange	V	V	V	Essential for gas conversion
	(6) Food preparation			V	After eco-system established
	(7) Wet oxidation waste manage.		V	V	Essential for eco-stabilities
	(8) Physico-chemical sodium extract.		V	V	Easily obtained with water recycle system
	(9) Nutrient chemical product.		V	V	Easily obtained with wet oxidation method
	(10) Low-G generator		V		Essential for testing gravity effect
EL-04	(1) Chetotaxis		V	V	After feasibility study is conducted
	(2) Organella		V	V	Same as above
	(3) Geotropism of plant	V	V		Essential for plantation
EL-05	(1) Fish and Animal production		V	V	Same as EL-01 (5)
EL-06	(1) Higher plant for foods	V	V	V	Same as EL-01 (1)
	(2) Algae for efficient gas exchange	V	V	V	Same as EL-03 (5)
	(3) Incineration waste management			V	After feasibility is studied
	(4) Physico-chemical sodium extract.		V		Same as EL-03 (8)
	(5) Nutrient chemical production		V		Same as EL-03 (9)
EL-07	(1) Fermentor			V	Same as EL-01 (2)
EL-08	(1) Cell culture			V	After feasibility is studied
	(2) Pathogen	V			Necessary to grow the bio-species
	(3) Protein rich flour separ.			V	After feasibility is studied
EL-09	(1) Plant cultivator	V	V		Essential for plantation
EL-10	(1) Bioreactor for food product.			V	After technology is established
EL-11	(1) Future experiment module			V	Future concept

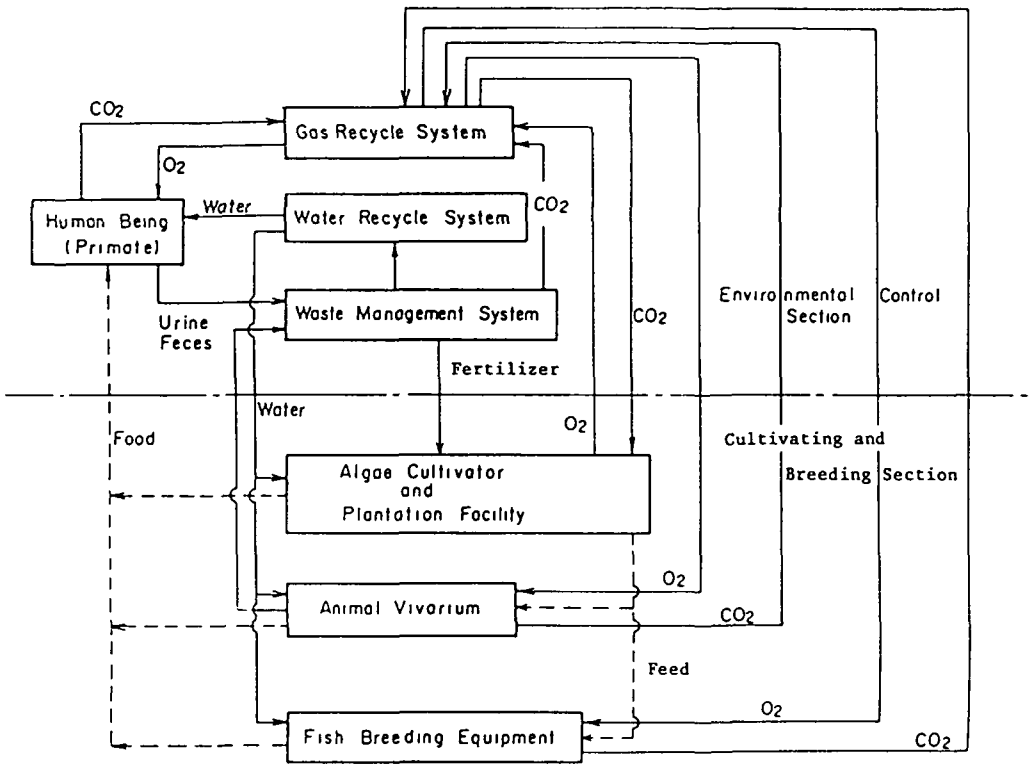

Fig. 1 CELSS Concept

photosynthesis and the possibility of propagation under the 0-G environment in Manned Space Station.

The second time phased mission which constitutes the dedicated mission is to be conducted during 1995 - 1998 for checking the possibility of a micro closed-ecology system using animals and fish instead of human beings, and the testings and evaluation of the non-biological system performance such as the gas recycle system, the water recycle system and the wet oxidation waste management system. Artificial gravity effects on the biological system are also to be evaluated.

In the last time phased mission, the main food supply and gas conversion from carbon dioxide to oxygen for a one man crew support are to be tested using the photosynthetic reaction of the plant and algae and the necessary animal protein production systems are to be evaluated using small animals and fish.

The experiment architectures for each mission are shown in Fig. 2. Due to this mission model, the preliminary design of all hardware necessary to conduct each time phased experiments and the investigation of integration methods to the space station have been studied and reported as shown in references 2, 3.

3. FUNCTIONS OF GAS RECYCLE SYSTEM

The appropriate quantities of oxygen, food and water should be continuously supplied for human beings and animals, and carbon dioxide and waste materials such as urine and feces should be taken away.

Both the oxygen and food necessary to animals, including human beings, are originally generated from the photosynthetic reaction of plants and algae using carbon dioxide and solar light. The carbon dioxide concentration on the earth is stabilized by the function of atmospheric circulation and the gas reservior function of sea-water. This 0.03% CO_2 concentration is not always appropriate for plant growth and it seems preferable to use higher concentrations, 0.3% and so on, for obtaining maximum growth rate. Therefore, the Gas Recycle System to be used in CELSS experiments has to have the ability to supply different CO_2 concentrations to the cabin and animal vivarium and the phytotron or higher plant cultivator.

In other words, the Gas Recycle System should have the following functions,

(1) to separate carbon dioxide and oxygen within the atmosphere provided from the cabin, the animal vivarium and/or the phytotron individually,

(2) to compress the separated gases such that carbon dioxide, oxygen and nitrogen can be stored in high pressure bottles,

(3) to release and supply the appropriate gases to the cabin, the animal vivarium and the phytotron individually through gas regulator manifolds.

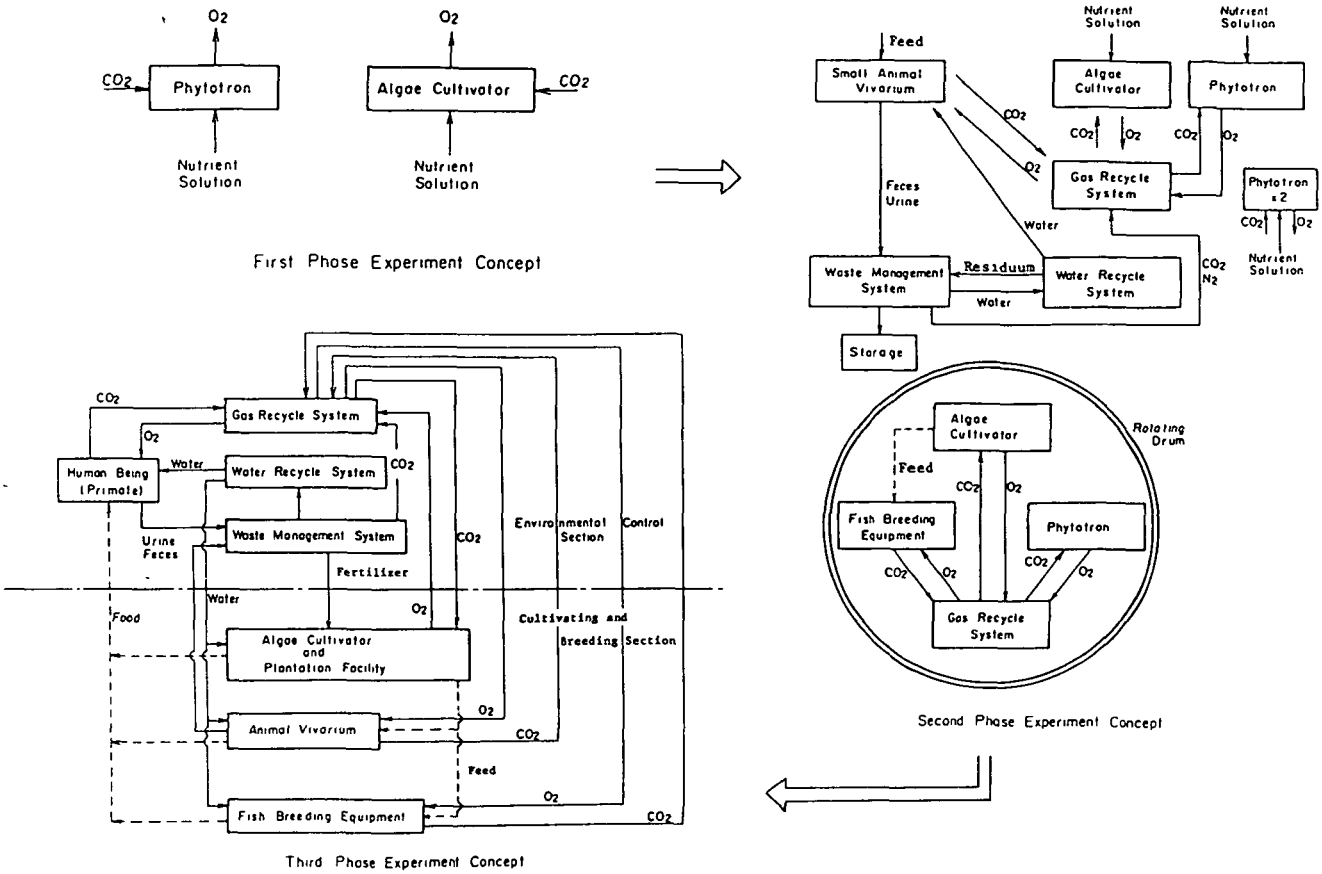

Fig. 2 Experiment Architectures of Each Mission

4. REQUIREMENTS FOR GAS RECYCLE SYSTEM

The various methods for separating each gas have been proposed and studied. (Reference 4-16)

Carbon dioxide gas separation, has typically used three methods, the molecular sieve, the hydrogen polarized cell and chemical absorption and desorption methods. Each has been discussed for application to CELSS experiments.

The molecular sieve method seems to be most reliable and has been used in Skylab, however dehumidification and the precise temperature and pressure control required for constructing the system using this method, would become more complicated. The hydrogen polarized cell method requires hydrogen to concentrate carbon dioxide, again the separation of hydrogen gas from the resultant gas and reduction of hydrogen gas are required.

The chemical absorption and desorption method seems to become more important since solid amine has been developed because of its regenerable and simple characteristics instead of LiOH, and the various applications are now being considered for use in the environmental control system in Space Station.

Therefore, based on this matter, this chemical method using the solid amine looks to be more preferable for adoption to the CELSS experiment hardwares.

As for the oxygen gas separation, three typical methods has been developed and used for various applications. One is again the molecular sieve method which has the same defect as mentioned above.

The second is the chemical absorption and desorption method using complex salt. This method has already been applied to testing the atmospheric control of the submarine and to the oxygen supply system for B-1 bomber, and this method is again preferable for being adopted to the CELSS experiment hardwares because of its simple characteristics.

The third one is the zirconia oxygen pumping method in which zirconia is used as a solid electrolyte for oxygen separation, this method also has simple characteristics and is preferable for being adopted to the CELSS experiment hardwares.

The gas recycle system capability to be designed has been assumed for supporting the respiration of a one man crew where the oxygen consumption per man-day is about 925 g/day and the carbon dioxide exhaust per man-day is about 1,130 g/day, corresponding to 30 lit./hr. of oxygen and 25 lit./hr. of carbon dioxide.

Therefore, the Gas Recycle System to be used in the CELSS experiments has to have the ability to supply these quantities of each gas.

5. DESIGN CONCEPT OF GAS RECYCLE SYSTEM

Fig. 3 shows the gas recycle sytstem functional diagram. Inlet gas is a mixture of O_2 (Oxygen), N_2 (Nitrogen), CO_2 (Carbon dioxide) and various trace contaminants.

At the filter, trace contaminants are removed.

CO_2 is separated and concentrated by a regenerable CO_2 absorber, and then, compressed and stored into the CO_2 gas bottle.

O_2 is also separated and concentrated by a regenerable O_2 concentrator, and, compressed and stored into the O_2 gas bottle.

Thus, inlet gas is separated and concentrated into CO_2, O_2 and N_2 gases.

Then, these gases are mixed properly and supplied to various utilities.

6. GAS ABSORPTION AND DESORPTION

In the gas recycle system, there are CO_2 and O_2 concentration processes. These are accomplished by two gas absorption and desorption processes, one is CO_2 absorption and desorption process using Solid Amine, and the other is O_2 absorption and desorption processes using Salcomine.

6.1 CO_2 Absorption and Desorption Process

CO_2 absorption and desorption is generally acomplished by using various kinds of amines, the chemical reaction could be described as shown below.

The solution of Ethanol Amine, such as Mono Ethanol Amine (MEA) and/or Diethanol Amine (DEA) absorbs CO_2 at the normal temperature and desorbs CO_2 at the high temperature, as indicated by the following reaction equation.

$$RR'NH + H_2O + CO_2 \underset{\text{(high temp.)}}{\overset{\text{(normal temp.)}}{\rightleftharpoons}} RR'NH_2CO_3$$

where $R = HOCH_2CH_2$, $R' = H$, for MEA
$R = R' = HOCH_2CH_2$, for DEA

This reaction has been directly applied to the CO_2 scrubbing unit in submarines. However, in space craft under the microgravity environment, such chemical agents seem not to be appropriate because of the difficulties of the gas and liquid separation.

Therefore, the solidification method of the amine has been studied and developed. Solid Amine consists of micro porous beads whose surface are coated with an amine. The substrate of beads is composed of a polymeric acrylic ester. (Reference 17,18)

Fig. 4 shows the CO_2 separating and concentrating system diagram. In this system, Solid Amine absorbes CO_2 at the normal temperature, and outlet gas from the solid amine canister is CO_2 lean gas.

Part of the CO_2 lean outlet gas flow returns to the cabin atmosphere, and the residual part of the flow is led to the next process.

When one canister becomes saturated with CO_2, the inlet flow is switched to the other canister and CO_2 absorption is continued in the new canister. The CO_2 saturated Solid Amine canister is heated and desorbs the CO_2. This CO_2 gas is led to the CO_2 compressor to be compressed and stored.

These Solid Amine canisters are used as absorbing, desorbing and precooling, respectively, and by the combination of canister cooling and heating, continuous CO_2 separation and concentration can be accomplished.

6.2 O_2 Absorption and Desorption

The O_2 absorption and desorption process is carried out using Salcomine. Salcomine (Bis(3-ethoxy salicyl aldehyde) ethylene diamine cobalt(II) - Fig. 5) absorbes O_2 at normal temperature and desorbes O_2 at the high temperature. (Reference 1)

Fig. 6 shows the O_2 separating and concentrating system diagram.

In this system, O_2 is absorbed into the Salcomine and N_2 gas comes out of the

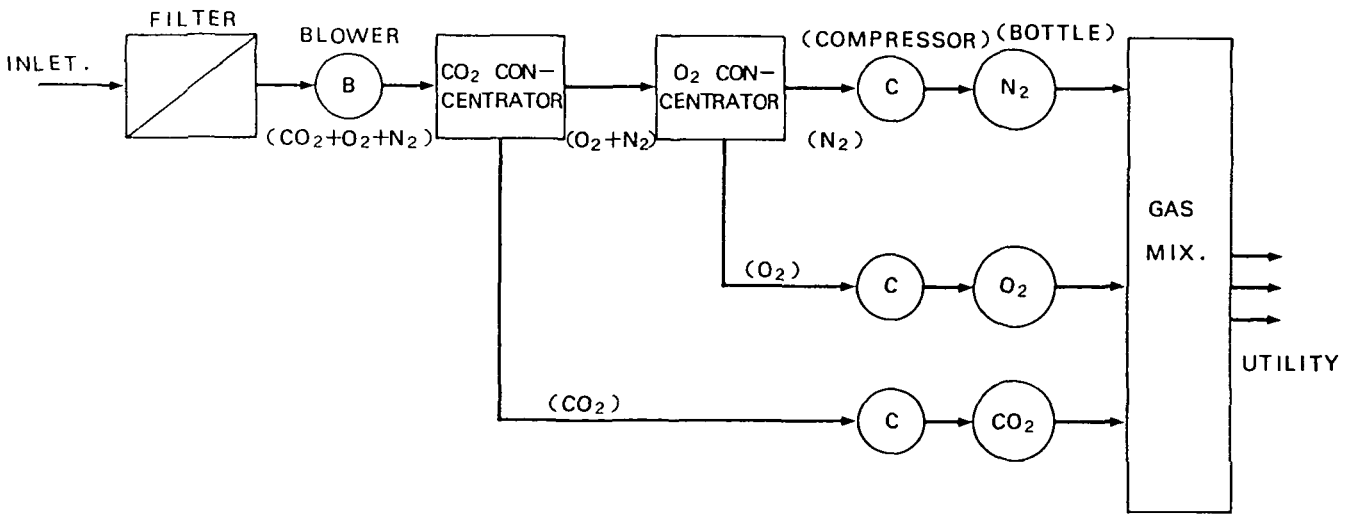

Fig 3 GAS RECYCLE SYSTEM FUNCTIONAL DIAGRAM

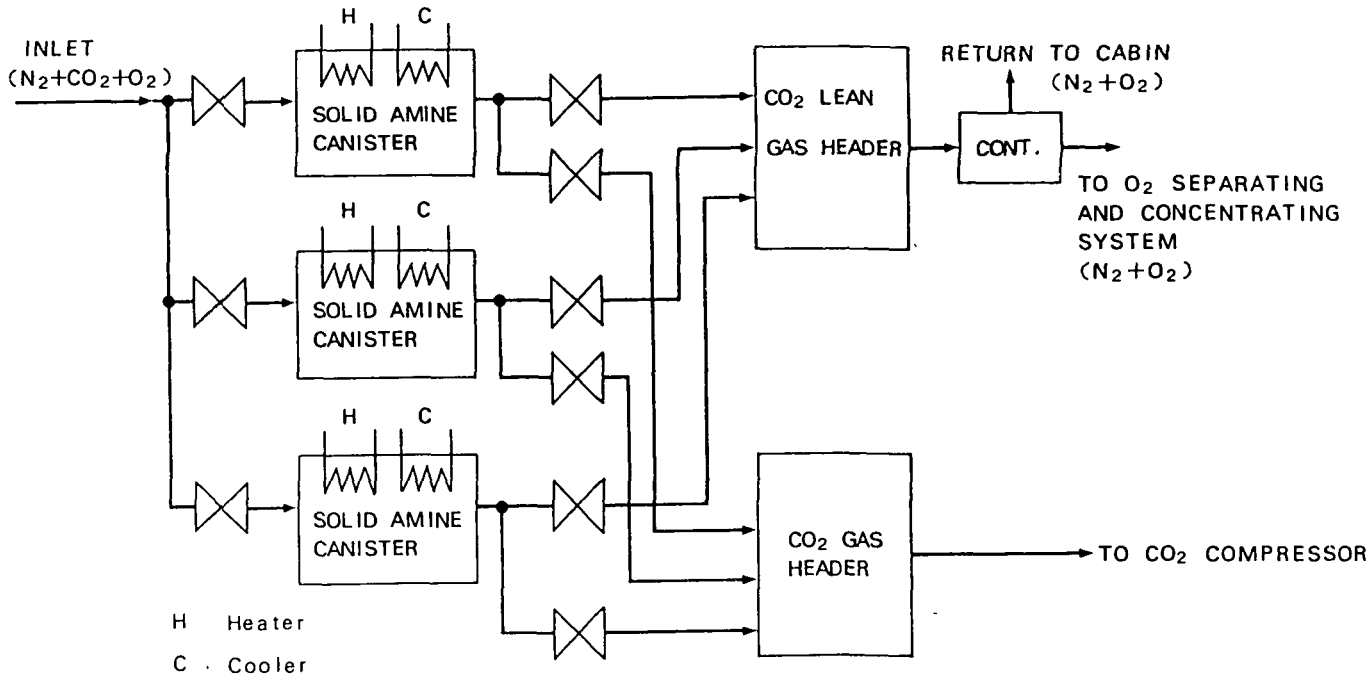

Fig 4 CO_2 SEPARATING AND CONCENTRATING SYSTEM DIAGRAM

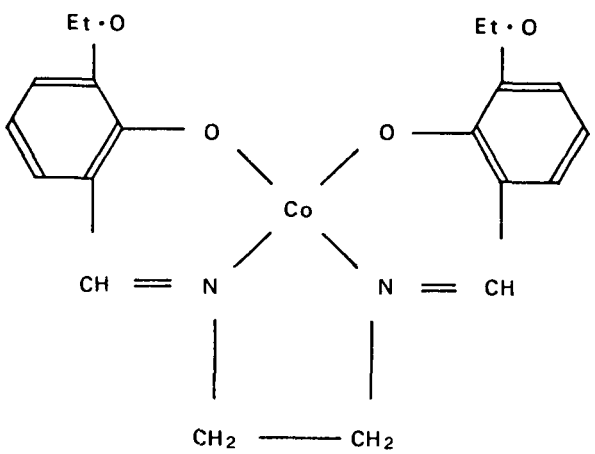

Fig 5 SALCOMINE

canister outlet. This outlet N_2 gas is led to the compressor to be compressed and stored.

When the Salcomine canister becomes saturated the absorption cycle is terminated and the desorption cycle is started, in this desorption cycle this canister is heated and desorbs O_2 gas. This O_2 gas is led to the gas compressor to be compressed and stored. Continuous O_2 absorption and desorption is carried out by means of cooling and heating three canisters alternately.

7. CONSIDERATION FOR DESIGN

Various CELSS experiment equipment will be considered in the design of the Gas Recycle System.

Phytotron (Plant cultivator) and RAHF (Research Animal Holding Facility) so called, the animal vivarium are considered.

Fig. 7 shows an example of Gas Recycle System application to the Phytotron and RAHF. In the RAHF, O_2 is consumed and CO_2 is exhaled by the metabolism of animals. In Phytotron, CO_2 is consumed and O_2 is exhaled according to the photosynthetic reaction of plants.

For stabilizing the O_2 and CO_2 gas concentrations inside the RAHF, O_2 is supplied from Gas Recycle System, and the CO_2 is pulled-out through the ventilator, this vented gas is mixed with the gas from the phytotron and the resultant gas is circulated through the Gas Recycle System and CO_2 is separated and stored for re-use.

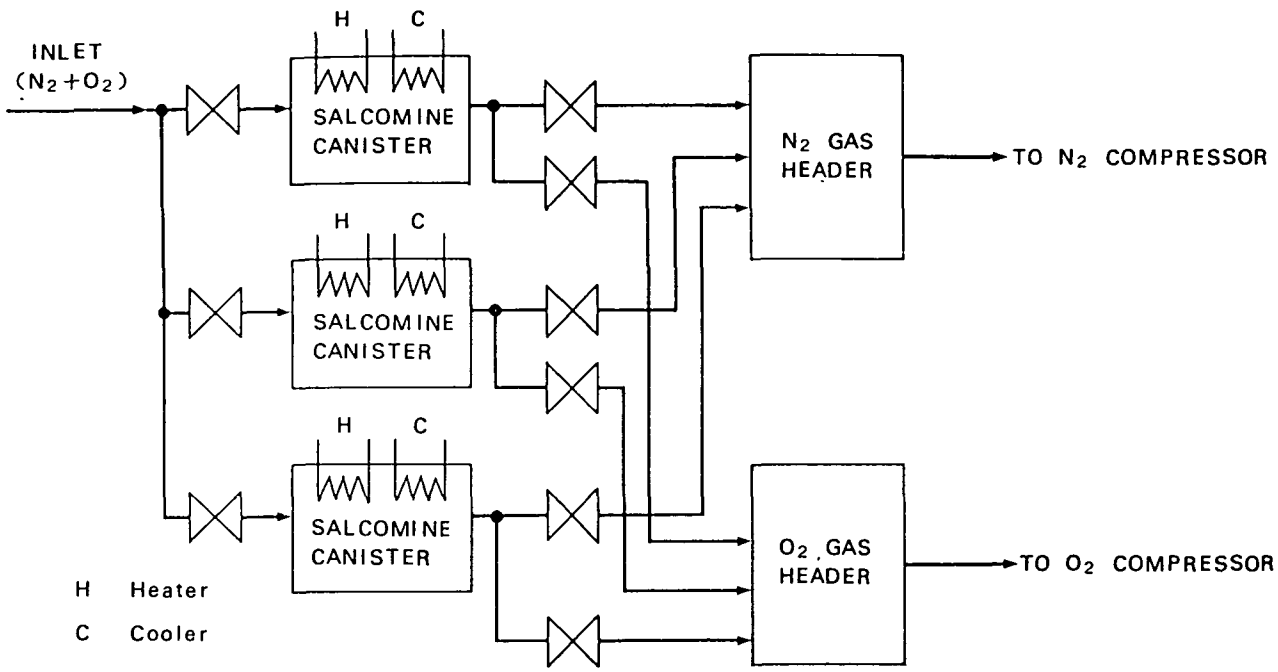

Fig 6 O_2 SEPARATING AND CONCENTRATING SYSTEM DIAGRAM

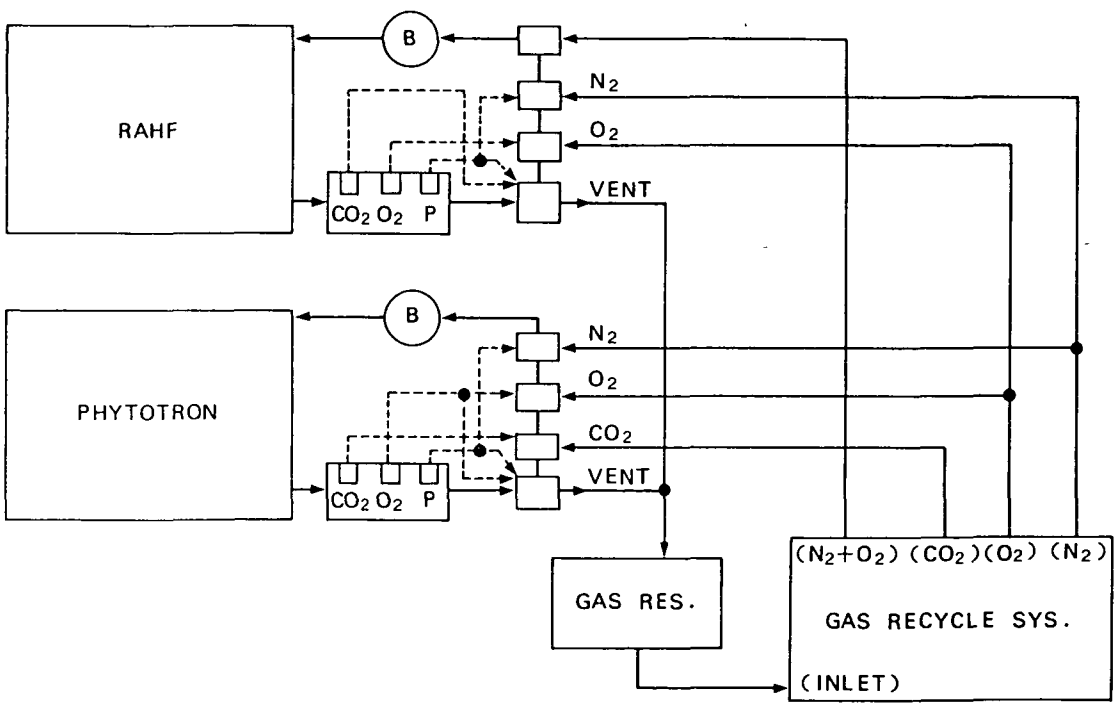

Fig 7 GAS RECYCLE SYSTEM APPLIED TO RAHF AND PHYTOTRON

According to the measurements of the pO_2 (partial pressure of O_2) and pCO_2 (partial pressure of CO_2) inside the RAHF the O_2 supply and gas venting is controlled.

The deviation of the total pressure (P) is compensated by the N_2 gas supply or gas discharging from the RAHF.

To the Phytotron, CO_2 is supplied from Gas Recycle System and the gas containing O_2 is also taken out by mean of the gas ventilator and this gas is again mixed with the gas from the RAHF and sent to the Gas Recycle System.

CO_2 supply is necessary to compensate the pCO_2 decrease caused by the photosynthetic reaction of plants.

The total pressure and the partial pressures are also controlled similar to the case of RAHF.

8. PRELIMINARY RESULTS OF DESIGN

The Gas Recycle System mentioned here comes from the design concept of carbon dioxide reduction system in the cabin for supporting human respiration. It may be possible to be able to improve the system for reducing power consumption. However the more detailed studies on the phytotron, the RAHF and so on will become necessary for this improvement.

8.1 Requirements

The design goal of this Gas Recycle System capability has been temporally given as shown in section 4. Namely this Gas Recycle System should manage 925 g/day of Oxygen and 1,130 g/day of carbon dioxide corresponding to 30 lit./hr. of O_2 and 25 lit./hr. of CO_2.

The operational pressure of the gas bottles is about 10 kgt/cm^{2}G.

For saving the gas compression energy, the lower pressure is better, but for making the compact design of system the appropriate high pressure such as 10 kgt/cm^{2}G is required.

Table 3 shows these requirements.

8.2 Gas Recycle System Block Diagram

Fig. 8 shows the Gas Recycle System Block diagram.

Inlet gas of 3,600 lit./hr. is drawn by the blower. At the filter containing activated chacoal and Hophalite (Carbonmonoxide (CO) oxiding catalyzer), the contaminants such as CO, odor and particles are removed.

At the CO_2 concentrater of Solid Amine (Solid Amine Canister) about 40 lit./hr. of CO_2 is obtained, and compressed to the pressure of 10 kgt/cm^{2}G and stored into the CO_2 gas bottles.

3,360 lit./hr. of the outlet gas (CO_2 lean) returns to the cabine atmosphere. The residual flow of 200 lit/hr. is led to the next process, Salcomine O_2 concentration.

At the Salcomine O_2 concentrator (Salcomine Canister), about 40 lit./hr. of O_2 is obtained and is compressed to the pressure of 10 kgt/cm^{2}G and stored into the O_2 gas bottles.

The residual flow, 160 lit./hr. of N_2 gas is compressed to the 10 kgt/cm^{2}G and stored into the N_2 gas bottles.

8.3 Gas Recycle System Configuration

Table 4 shows the list of principal components of the Gas Recycle System.

Table 3 GAS RECYCLE SYSTEM REQUIREMENTS

No.	Item	Unit	Value	Remarks
1.	Flow Rate			
	Oxygen	1/h	30	for one man
	Carbon dioxide	1/h	25	Life Support
2.	Purity			
	Oxygen	%	above 90	
	Carbon dioxide	%	about 90	
	Nitrogen	%	about 90	
3.	Operating Pressure	kgt/cm^{2}G	10	

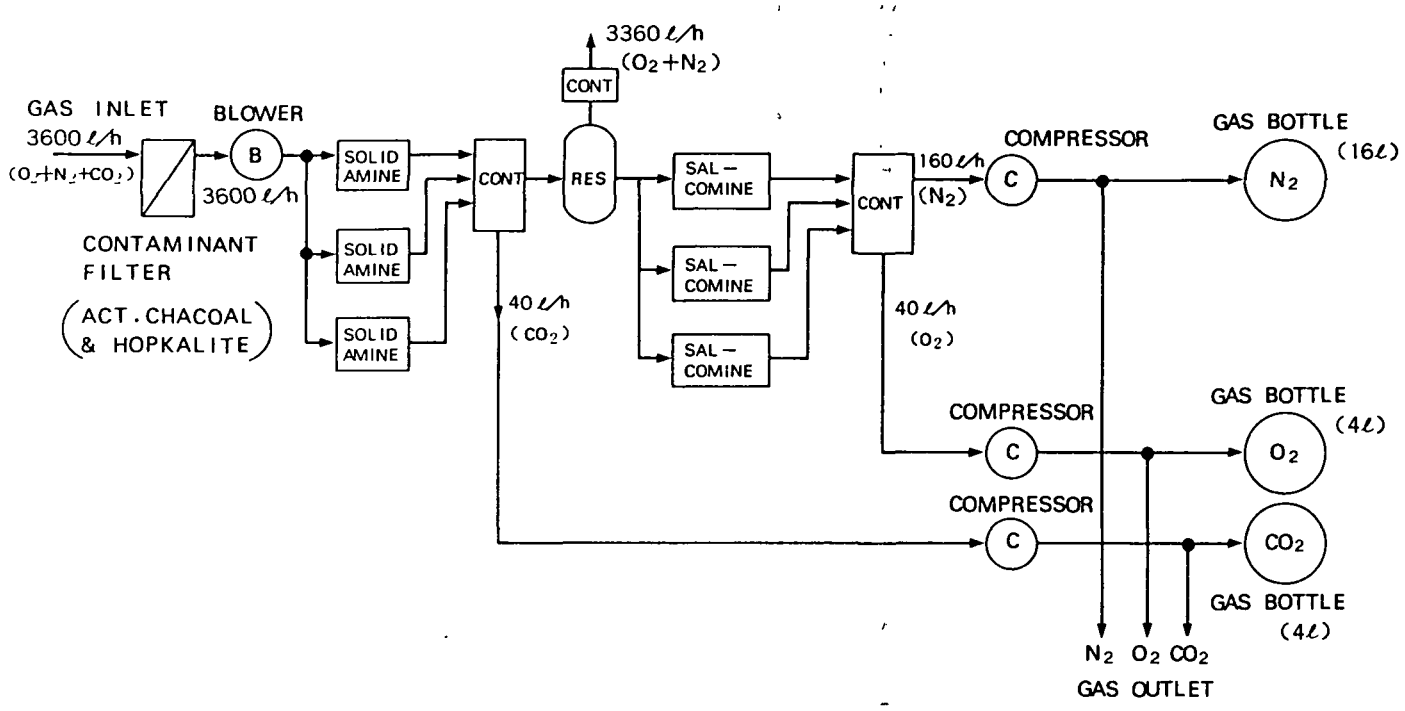

Fig 8 GAS RECYCLE SYSTEM BLOCK DIAGRAM

Table 4 GAS RECYCLE SYSTEM COMPONENTS LIST

No.	Name	Quan.	Particulars	Man (kg) @	Man (kg) Total	Power(kw) @	Power(kw) Total	Remarks
1.	Reservor	1	30 lit.	3	3	-	-	
2.	Filter	1	Act. Chacoal Hopkalite etc.	2	2	-	-	
3.	Blower	1	3,600 lit./h	6	6	0.1	0.1	
4.	Sol.Amin Unit	3	3,600 lit./h	4	15	0.5	0.5	
5.	Reservor	1	10 lit.	2	2	-	-	
6.	Salcomine Unit	3	20 lit./h	3	9	0.5	0.5	
7.	Compressor	1	160 Nlit./hx10k	6	6	0.1	0.1	N_2
8.	Do.	1	40 Nlit./hx10k	4	4	0.05	0.05	O_2
9.	Do.	1	40 Nlit./hx10k	4	4	0.05	0.05	CO_2
10.	Gas Bottle	1	16 lit.x10k	3	3	-	-	N_2
11.	Do.	1	4 lit.x10k	1	1	-	-	O_2
12.	Do.	1	4 lit.x10k	1	1	-	-	CO_2
13.	Controler	1	-	16	16	0.1	0.1	
14.	Valve Pipe	-	-	-	50	-	-	
15.	Cable etc.	-	-	-	30	-	-	
16.	Frame	-	-	-	50	-	-	
	TOTAL				202		1.40	

The total mass of this system and the electric power consumption are estimated about 202 kg and about 1.40 kw respectively.

Fig. 9 shows the configuration of the Gas Recycle System. The components of the Gas Recycle System will be assembled within the space of the Single Rack of the SPACE LAB.

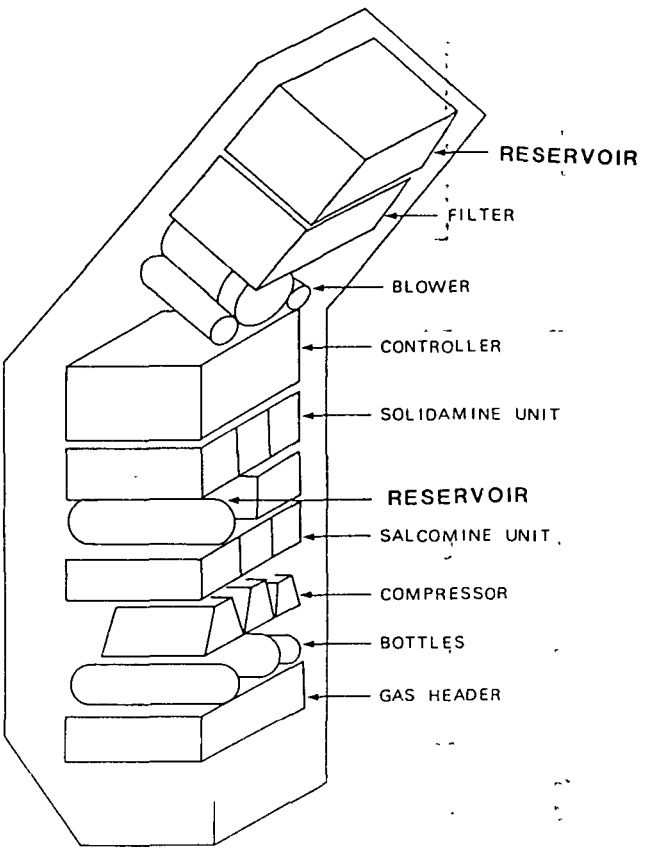

Fig 9 GAS RECYCLE SYSTEM CONFIGURATION

9. Conclusion

Through the concept studies for CELSS experiments in Space Station, the following results had been obtained.

(1) The guideline of the CELSS technology research and development has been elucidated through the time phase mission sets as the strawman model,

(2) The development feasibility of the various hardware necessary to conduct the CELSS experiments in each time phased mission and the preliminary interface requirements for each mission sets has been clarified through the concept design studies.

In spite of these fruitful results, many problems to be solved for developing hardware have been found through these studies.

As for the Gas Recycle System, the next two problems seem to be very important for establishing the stability of the system.

(3) The degradation mechanism of O_2 absorber agent such as complex salt should be tested and analyzed through bench tests and if the degradation characteristics are not sufficient a more stable agent should be developed.

(4) For establishing complete gas recycle in the CELSS, the balance between the respiration quotient of hetetotroph and the assimulation quotient of autotroph should be established within a definite period of time, the possibility for keeping this balance with the gas recycle system should be tested and checked through ground based experiments, if impossible, the additional equipment for keeping the balance should be introduced as a subsystem in the CELSS hardware.

These studies had been conducted under the support of many researchers belonging to the CELSS research group.

The Authors greatly appreciate their support.

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APPENDIX

Tables

1 Proposed Theme at the First Japanese Space Station Simposium
2 The Proposed Research Items and the Necessary Time Span for Hardware Development
3 Gas Recycle System Requirements
4 Components List of Gas Recycle System

Figures

1 CELSS Concept
2 Experiment Architecture of Each Mission
3 Gas Recycle System Functional Diagram
4 CO_2 Separating and Concentrating System Diagram
5 Structure of Salcomine
6 O_2 Separating and Concentrating System Diagram
7 Gas Recycle System Applided to RAHF and Phytotron
8 Gas Recycle System Block Diagram
9 Conceptual Configuration of Gas Recycle System

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Utilization of Membranes for H_2O Recycle System

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ABSTRACT

Conceptual studies of closed ecological life support systems (CELSS) carried out at NAL in Japan for a water recycle system using membranes[1] are reviewed. The system will treat water from shower room, urine, impure condensation from gas recycle system, and so on. The H_2O recycle system is composed of pre-filter, ultrafiltration membrane, reverse osmosis membrane, and distillator. Some results are shown for a bullet train of toilet-flushing water recycle equipment with an ultrafiltration membrane module. The constant value of the permeation rate with a $4.7m^2$ of module is about 70 l/h after 500h of operation. Thermovaporization with porous polytetrafluorocarbon membrane is also proposed to replace the distillator.

WHEN A SPACE STATION is constructed as a permanent facility with long-term human habitation food, water and oxygen are indispensable. Operation cost must be kept as low as possible by reducing materials transported to and from the earth.

If the human wastes could be recovered and regenerated to produce food, water and oxygen a significant amount of mass transport from the earth could be reduced and the human wastes would not be returned back to the earth as on current manned flights.

On the earth, surface water is constantly evaporated by solar heat and recycled to the surface again as forms of raindrops and snow. In the space station, there is no such natural water recycle system available, so an artificial water recycle system is important and necessary for the purpose to establish a closed ecological life support system(CELSS).

2ND PHASE SPACE MISSION

The use of a CELSS in space habitats is the reason why a compact water recycle system is scheduled in the Japanese NAL study to test its performance under 0G condition in the second phase of a series of space missions. The system is composed of two main parts, a shower water recycle loop and a water purification loop. The shower water from the bath room, which is typically 20 liters per a shower, is fed into high pressure pump through a water filter, and then purified by a reverse osmosis (RO) membrane moduie. Purified water is stored in a tank and will be supplied for the successive use in a shower, and impurity such as residual condensation from the spacecraft atmosphere will be also sent to the water purification loop.

Table 1 summarizes the tentative operational specifications for the shower water recycling loop, and required measurement items for the system operation are indicated in Fig.1 and listed in Table 2.

The drainage from the shower water recycle system is introduced into the water purification system, together with other water drainage, urine and condensed expiration water. Accordingly, the system is required to have the capacity to handle the items in Table 3. The system will purify water by the integrated ultrafiltration membrane (UF), reverse osmosis membrane and distillator.

The entire system block diagram is shown in Fig.2 and the system design goal are tabulated in Table 4. In Table 5 monitoring parameters for the system operation are summarized.

To satisfy space station safety requirements, the system should be operated with an adequate interlock circuit. Particularly, the distillator should be designed with enough hazzard protections. Additionally, a vital area of research is determining the stability of membranes and filters over time, and monitoring the amount of residue in the recycled water.

The distillator is operated with a batch

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process mode, and contains a small centrifugal phase separator, heater, and air cooler for water condensation.

The residual impurity solution from the ultrafiltration module and distillator will be stored in a tank for a further processing by a waste management system.

The total system should be made compact to be contained in a small box so that it does not occupy a large space in space station. In Table 6, specification of the water recycle apparatus are listed. The schematic of the entire system is shown in Fig 3, and a three dimensional picture of the system is shown in Fig 4.

SOME RESULTS ON ULTRAFILTRATION MEMBRANE[2)]

For the purpose of obtaining reliable data for system design to treat waste water containing urine, feces and other solids, a brief summary of results obtained with an ultrafiltration membrane module is shown which is contained in toilet-flushing water recycle equipment specially designed for bullet train which is now on a development and demonstration stage. The ultrafiltration module has 4.7m^2 of surface are and is a hollow fiber type 0.8mm^{O} x 1.4mm^{O} x 1000mmL and made of polyacrylonitrile.

The toilet will serve 174 persons and will be used 26 times per hour. The total amount of volume of urine and feces per hour is estimated as 7 liters. The design specifications are listed in Table 7, and a flow diagram is shown is Fig 5.

Used water is pumped through a rotating strainer with 0.6mm slits and fed to a prefilter with 75 m screen and a rubber scraper. The filtered water is fed to ultrafiltration modules. Through which the permeation rate is about 200 l/h at the beginning but gradually decreared and reaches a stable value of 70 l/h after 500 hours of operation. The membrane life now obtaine is about one and a half years. The average values of the quality of permeate are listed on Table 8.

THERMOVAPORATION IN PLACE OF DISTILLATOR

Reliable data of the performance of reverse osmosis module will be obtained in the near future. Permeate through reverse osmosis membrane will be used as plant cultivation water after activated carbon treatment and UV-light sterilization.

Thermovaporization was proposed recently to replace the distillator for water for small animals. The membrane is composed of porous polytetrafluorocarbon. The pressure in the permeate side of the membrane is 50mmHg and temperature will be kept 20^{O}C. Membrane area needed is estimated as about 0.3m^2 to obtain distilled water at a rate of 5 l/6h.

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Table 1 Design Goal for Shower Water Recycle System

Item	Description
Water Recovery Ratio	>95%
Pressure Difference	<60 atm.
Capacity	>0.5 ton/day
Operating Time	≃5 Hr/day
Power	kW

Table 2 Monitoring Parameters for the Shower Water Recycle System

Location	Measurements
Pump Inlet	Temperature ,Pressure
RO Filter Inlet	Eletrical conductivity,Pressure
RO Filter Recirculation Loop	Flow rate
RO Filter Outlet	Electrical conductivity,Pressure

Table 3 Capacity Requirement for the System

Item	Amount
Urine	1.8 lit./man-day
Shower Drainage	1.0 lit./man-day
Expiration	1.2 lit./man-day
Other Drainage	1.0 lit./man-day
Total	5.0 lit./man-day

Table 4 System Design Goals

Item	Design Goals
Recovery Ratio(UF)	>90%
Pressure Difference(UF)	≃2 atm
Recovery Ration(RO)	>50%
Pressure Difference(RO)	<60 atm
Capacity	>5 lit./day
Operating Time	19 Hr/day

Table 5 Monitring Parameters for the Water Purification System

Location	Measurements
UF Filter Inlet	Electrical conductivity, Transparency,Pressure, Temperature,Urine content Biological oxygen demand (B.O.D.),Chemical oxygen demand(C.O.D.)
RO Filter Inlet	Electrical conductivity, Pressure,Temperature

Table 5 (continue)

RO Filter Recirculation Loop	Flow rate, Pressure
RO Filter Outlet	Electrical conductivity, Pressure
Distillator	Pressure, Temperature

Table 6 Specification of the Water Recycle System

Item	Specifications
Dimensions (mm)	450W x 1490H x 610D
Weight (kg)	about 330
Power (kW) Requirement	2.4
Membrane Filters	RO: 0.5 ton/day 0.5 m^2 Membrane area 20-40 atm Press. Diff. UF: 5 lit./day 0.5 m^2 Membrane area 2 atm Press.Diff.
Distillator	5 lit./Hr (Max.) Centrifugal Phase Separation

Table 7 Design Specifications for Toilet Flushing Water Recycle Equipment for Bullet Train

Item	Specifications
Tank for flushing Water	200 l (overflow)
Tank for used water	350 l (initial volume of water 150 l)
Ultrafiltration	70 l/h/module x 3 mod.
Prefilter	130 l/h/module x 2 mod.
Main pump	220 l/min, 22.5 m, 1.5kW
Flushing pump	27 l/min, 2 m, 0.4 kW
Maximum power	2.1 kW

Table 8 Quality of Permeate by UF

Item	Amount
BOD (mg/l)	257
COD (mg/l)	22.8
Suspended Solid (mg/l)	4.5
E.Coli	not detected
Oder (JIS K0102.10.2)	4.2
Transparency (JIS K0102.10.3)	16
pH	8.7
Color	white - yellow

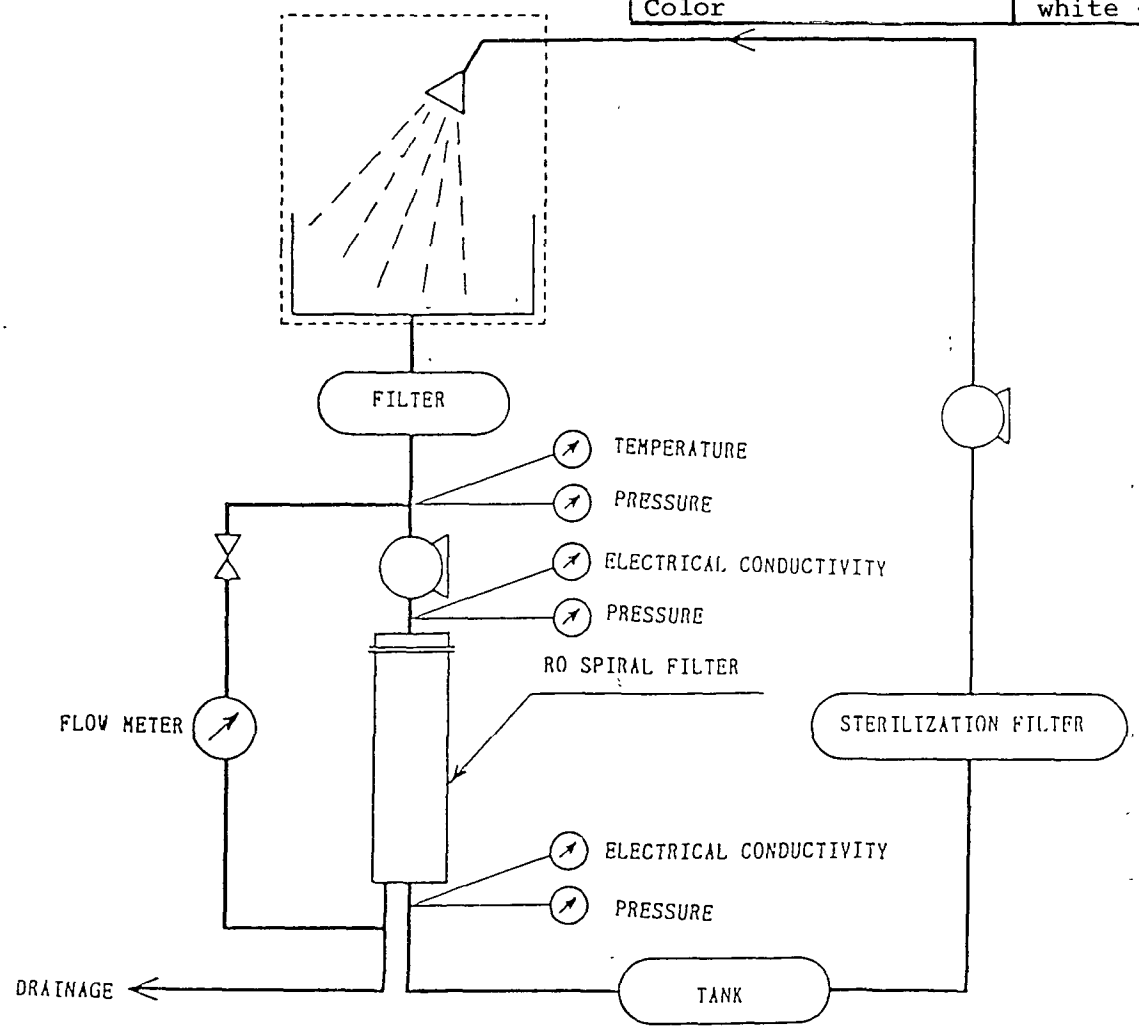


Fig. 1 Shower Water Recycle Loop

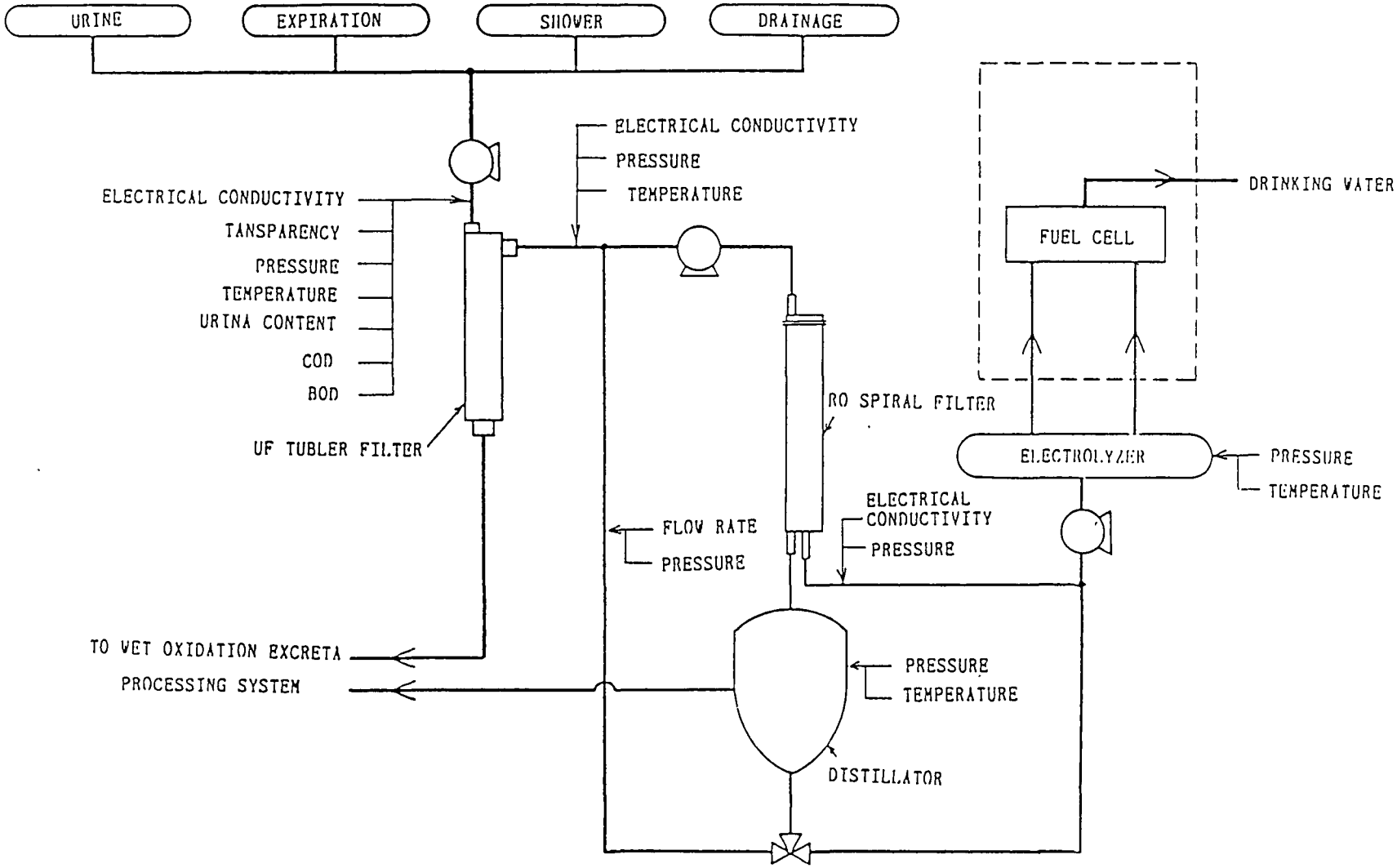
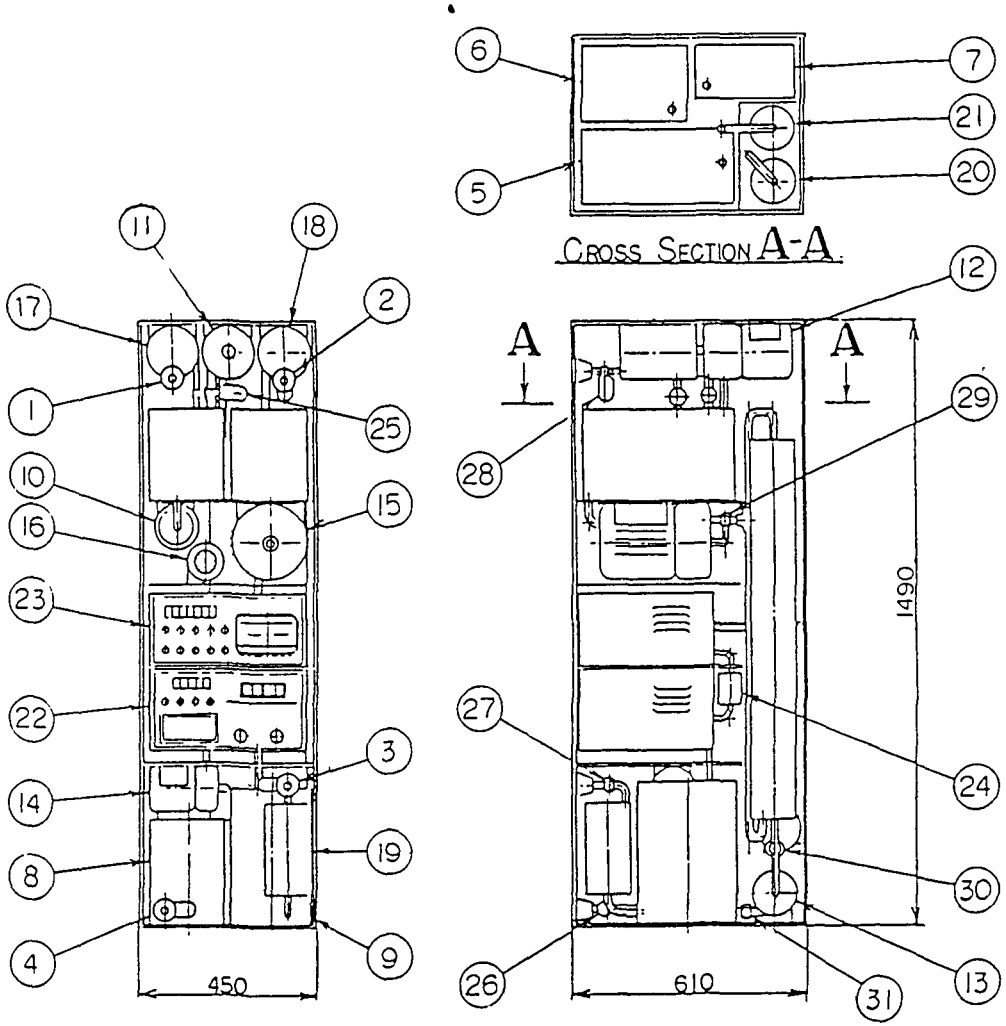


Fig. 2 Water Purification Loop



NO	TITLE
1	USED WATER/URINE INLET
2	SHOWER DRAINAGE INLET
3	SHOWER WATER OUTLET
4	RESIDUAL LIQUID OUTLET
5	TANK
6	TANK
7	TANK
8	TANK
9	TANK
10	PUMP
11	PUMP
12	PUMP
13	PUMP
14	PUMP
15	HIGH PRESSURE PUMP
16	BLOWER
17	FILTER
18	FILTER
19	STERILE FILTER
20	RO SPIRAL FILTER
21	UF TUBLER FILTER
22	DISTILLATOR
23	ELECTROLYZER
24	NaCl REMOVER
25	VALVE
26	VALVE
27	VALVE
28	VALVE
29	VALVE
30	VALVE
31	VALVE

Fig. 3 Composition of Water Recycle System

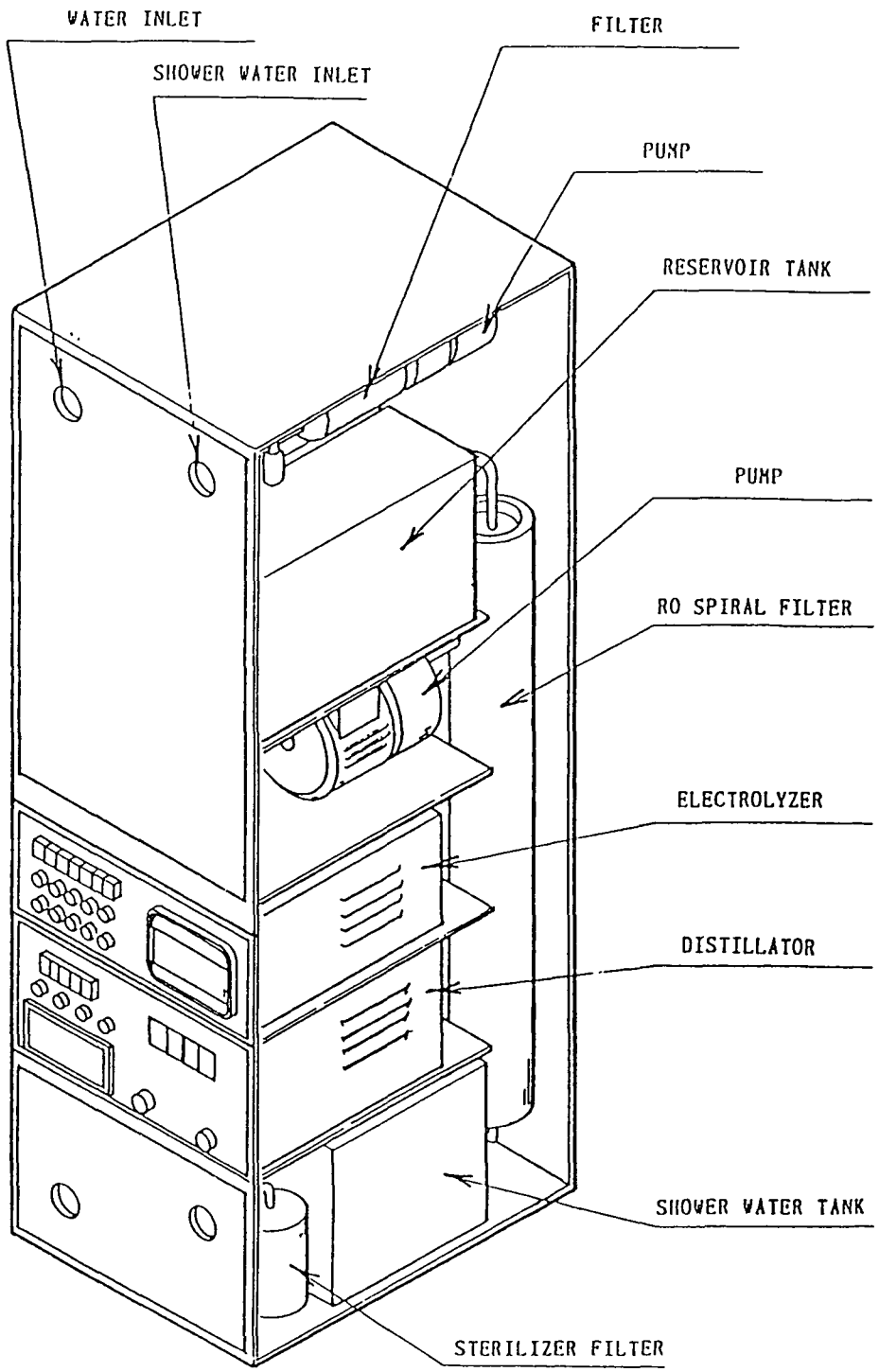


Fig. 4 Configuration of Water Recycle System

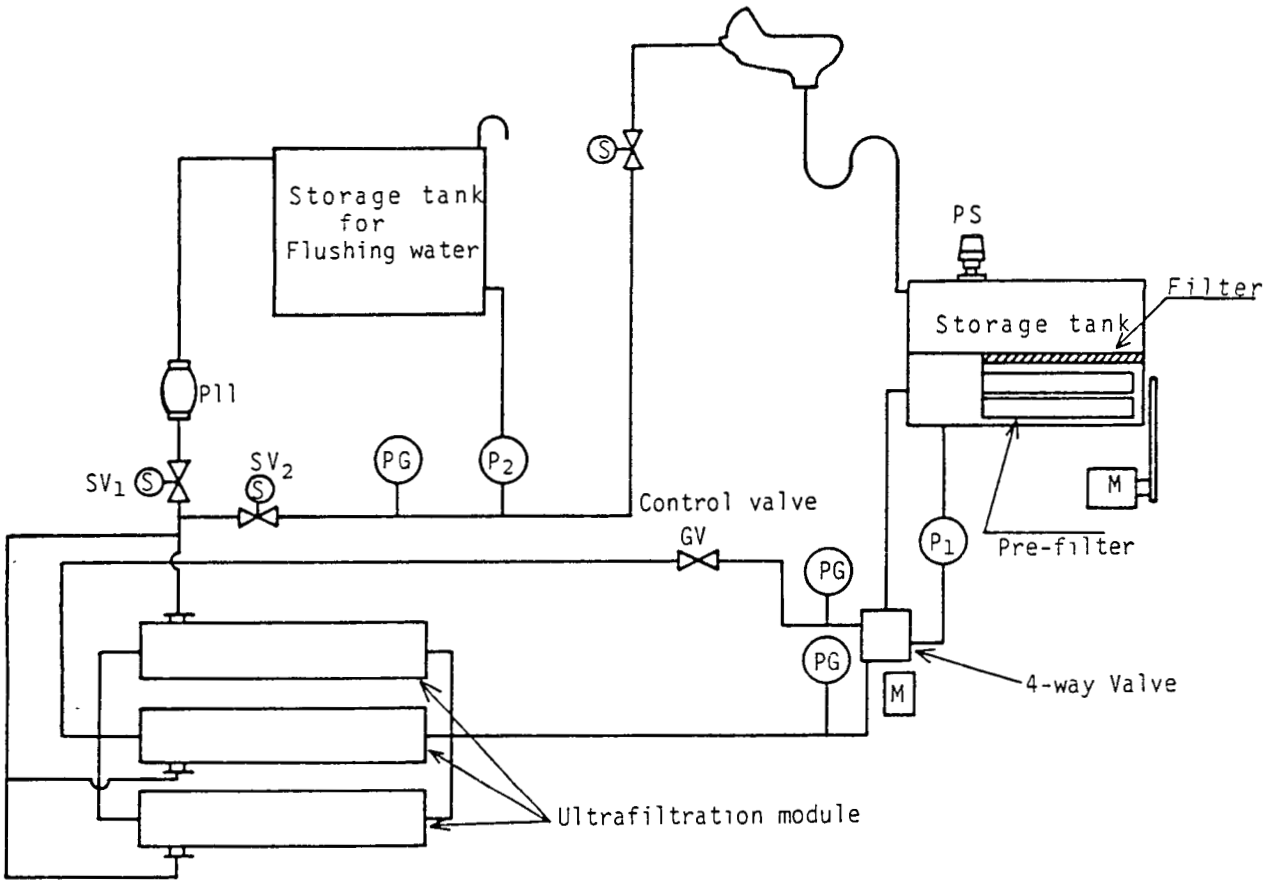


Fig.5 Flow diagram of toilet flushing water recicle equipment for bullet train

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The $C_2$3A System, an Example of Quantitative Control of Plant Growth Associated with a Data Base

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ABSTRACT

The architecture of the $C_2$3A (Chambres de Culture Automatique en Atmosphères Artificielles) system for the controlled study of plant physiology is described :

1) Modular plant growth chambers and associated instruments (I.R. CO_2 analyser, Mass spectrometer and Chemical analyser).

2) Network of frontal processors controlling this apparatus

3) Central computer for the periodic control and the multiplex work of processors. It also concentrates the data, obtained from processors, and stores them in long term data base.

4) Network of terminal computers able to ask the data base for data processing and modeling .

Examples of present results are given : growth curve analysis, study of CO_2 and O_2 gas exchanges of shoots and roots and daily evolution of algal photosynthesis and of the pools of dissolved CO_2 in sea water.

This system is extremely useful to continue progress in agricultural research. Another application is in Controlled Ecological life Support Systems (CELSS) for space habitats.

AGRONOMY AND ECOLOGY are studies of macrosystems that require basic knowledge from the elementary disciplines of molecular biology, biochemistry, and cellular physiology for the study of botanical systems. None the less, whole plant physiology remains a necessary field of research because the study of the whole system cannot be directly, nor even necessarily deduced, from sublevel or microscopical characteristics, althought these cellular parts are under the control of the genetic program and large-scale environmental factors. This synthesis shows properties which largely remain to be discovered. The integration of studying plant micro-processes with respect to the larger environment is necessary to understand the function of plants and their communities.

The lack of indepth knowledge of plant behaviour becomes both more obvious and more crucial when studying models of whole systems, for example, to predict the long term effect of CO_2 increase on vegetation, (1) or to cultivate plants in chambers under totally artificial conditions in complex ecological cyles such as for the Controlled Ecological Life Support System (CELSS) program (2), suggested for the economical habitation of space stations over periods ranging from months to years.

Conversely, studies at the microscopic level have most frequently been initiated, oriented ans stimulated by observation of macroscopical phenomena. That tends to be forgotten and the interest in whole plant studies has decreased, at least in France, to the profit of new areas like molecular biology.

WHAT IS THE CAUSE of this disinterest, in spite of the needs mentioned above ? It could be that the traditional methods of integrated physiology do not fulfill these needs, or that they have already given their best results using past methodologies, and that answers they can bring now do not justify the experimental effort involved.

Among the many possible reasons for the difficulty of indepth studies in plant physiology , the first is the exagerated diversity of plants studied in plant physiology (many in the juvenile stage), and a seemingly infinite variety of often insufficiently defined experimental conditions. This leads to a multiplication of studies, without allowing a systematic comparison of results and progress (3). Still more important is the separation of scientific specialists.

Studying a complex system where many organs, functions, and a climatic or other trophic factors are interdependent, progress in understanding cannot result without systematically studying the correlations

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between and among the various functions and organs.

ANOTHER DIFFICULTY is that the time factor is rarely mastered. The plant system is growing and evolving, it is shaped by the history of its environnent and keeps a memory of it (for example : the stress acclimation). So the constant monitoring and control of all kinetic data is necessary for understanding these processes and relating long-term to short-term responses, long-term responses being the most interesting to agronomists.

This explains why we developed a new experimental system with the purpose of studying plants as integrated systems, our main concern being photorespiration. The equipment should enable us to :

1) Realize an environment approximating natural conditions, especially light conditions.

2) Simultaneously measure the main physiological functions, by non-destructive methods.

3) Quantify the relations between shoot and root metabolism.

4) Analyse time effects by short-term (hour, day) and long-term (month, season) observations, by means of high capacity data files.

5) Relate global behaviour of the system to microscopic characteristics.

THESE AIMS MAY APPEAR AMBITIOUS. Experience has shown that they are attainable using automation and computers. It is enough to apply to plant research the methods common in medical or physical research. Several Laboratories have constructed equipment with similar objectives. The most important to our knowledge is the SPAR system (4). The controlled-environment chambers of Jones et al (1984) is also a good example of the equipment built in American universities for the study of increased atmospheric CO_2 on crop canopies or natural vegetation.

A first version of our system, named C_23A, developed since 1974, has been described (6). It has been improved and the computer system is being rebuilt on a more decentralized mode. We shall use this opportunity to present the new version, its principles and functionning. Experimental possibilities will be illustrated by typical results.

I - PRINCIPLES OF THE C_23A SYSTEM

(for : Chambers for Automatic Cultivation in Artificial Atmospheres).

The general idea is to achieve a direct recording of physiological activities of the plants in culture and store the data in files fit for immediate or delayed use (fig. 1).

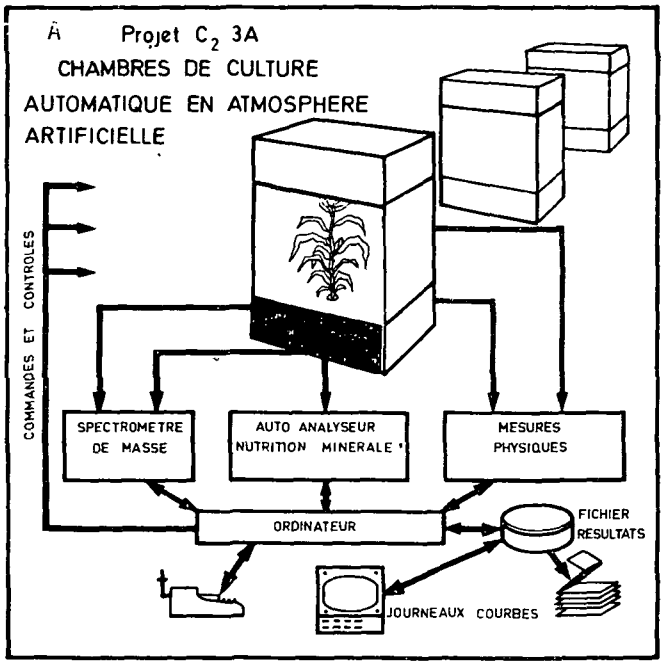


Figure 1 (A) General Principle of C_23A system.

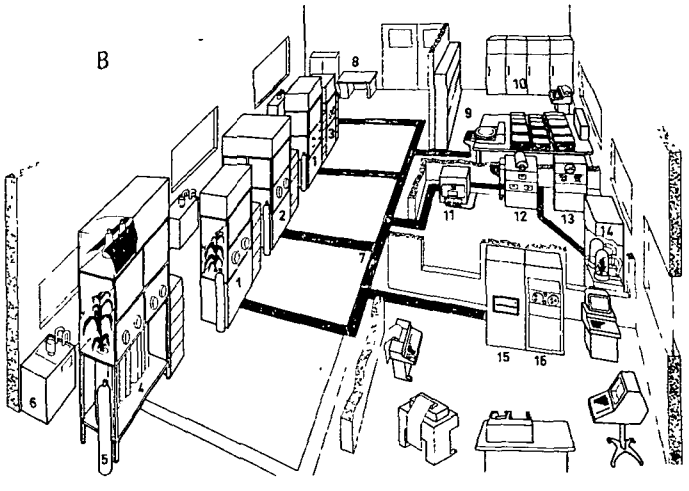


Figure 1 (B) Experimental area : (1, 2, 3) Growth chambers. (4) Twin chambers (5) CO_2 supply. (6) cool water supply. (7) Network conduit : circuits to analyse, monitor and control gas and solutions. (8) Little workshop. Specialized laboratories : (9) Chemical analysis with (10) torage of samples in deep-freeze chambers. (11) Centralized analysis of CO_2. (12) (13) Quadrupolar mass spectrometer. (14) Mini-chambers (4 to 40 l) iters (15) Computer control with (16) board of magnetic disks.

To monitor and direct the growth of plants we associate :

- leakproof culture chambers of various dimensions,
- automatic gas analysis (mass spectrometer and CO_2 infrared analyser) and regulation systems,
- automatic chemical analysis (Technicon),
- a computer system that drives in real time the abovementioned apparatus, provides visual or graphic control and stores all data in short-term and long-term files.

The physiological activities, available by measurements of matter exchanges between the plant and its environnents, are : photosynthesis in O_2 and CO_2, photorespiration measured by $18O_2$ uptake, respiration of shoots and roots, transpiration, nutrition by mineral uptakes.

II - THE CULTURE CHAMBERS

The system comprises three types of chambers, which differ notably, adapted to the study of canopies, isolated plants with a separate root compartment, and acquatic plants.

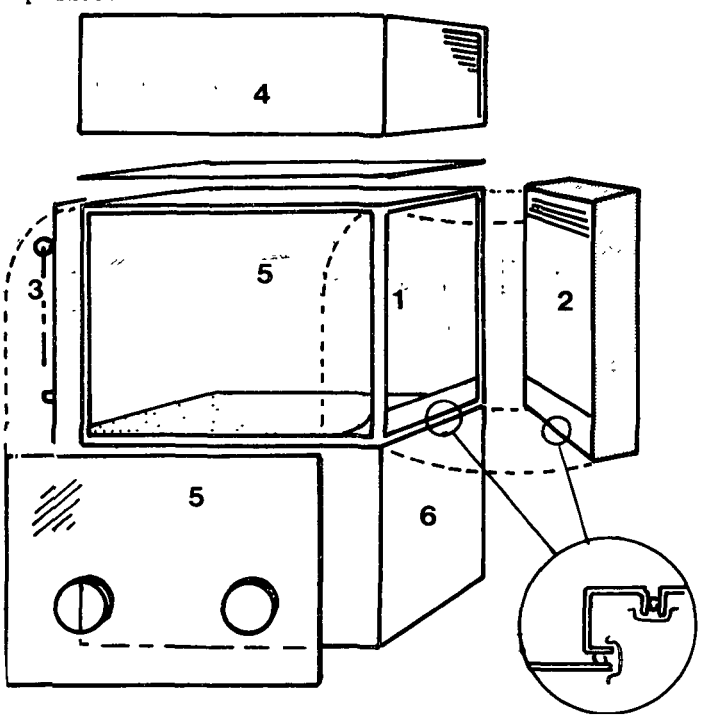


Figure 2A

Culture chambers are made by assembling on a frame unit (1) : an air conditioning unit (air-water exchanger) (2), a gas regulation unit (3), a light unit (4), front and rear panels (5) , and a stand (6). All assemblies are made with clamps and are easily disassembled. A nutrient solution supply unit can be placed on the stand.

THE LARGE CHAMBERS for the study of canopies comprise a modular frame (1 x 0.6 x 1.2 m units), a lighting module, an air conditioning unit, a gas regulation unit, and detachable front and rear panels. Several frame units can be easily assembled for making various volumes or twin chambers (figure 2). The junction between chambers by means of clamps and rubber gaskets ensures the same air-tightness as would O-rings. To optimize the simulation of a crop canopy, the side and rear panels of the chamber are covered with polished aluminium, and the front panel is coated with a semi-reflecting film, so as to reduce the border effect

AIR CONDITIONING UNIT. Dry bulb temperature is regulated by the variation of the rate of air flowing through a large area (18 m^2) heat exchanger. This copper-foil exchanger receives water at the temperature of the desired dew-point.

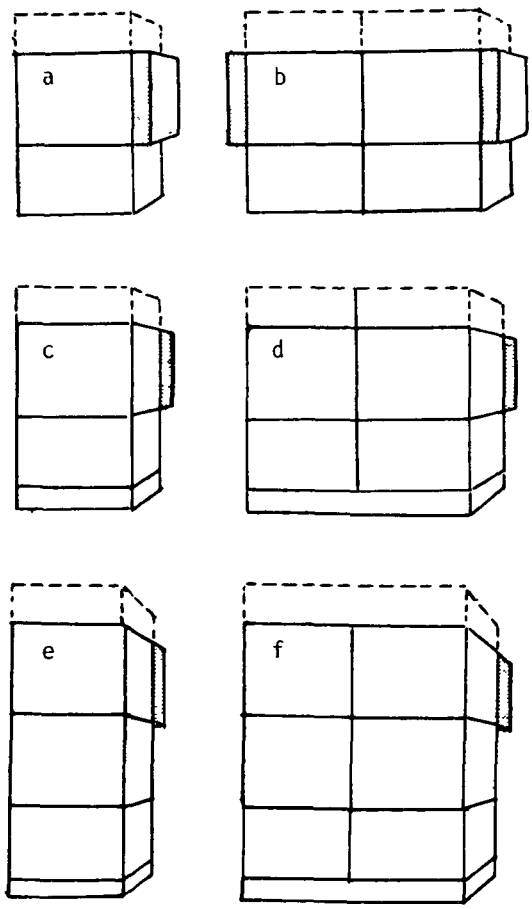


Figure 2B

Example of realization. The growth chambers can be made of 1 module (a,c), 2 modules placed side-by-side (b,d) or single-stacked (e), or made of 4 modules (f). According to need of irradiance two units to control temperature and humidity can be attached to the module (on the rear) (c,d,e,f). The combinations a,b,c,f are in operation in our laboratory.

This has two advantages : hygrometry is very accurate, and the continuous weighing of the water condensed by the exchanger measures the transpiration of the plants.

THE SMALL CHAMBERS (fig.3) from 1 to 40 liters in volume are adapted to the study of one whole plant. They include a metal base containing the heat exchanger.

The plant pot or a flask for hydroponic culture can be fitted below the metal base, and a glass container fits over the top of the plant. A tight seal is ensured by O-rings.

The root and shoot atmospheres are separated by a plastic plate with a hole for the plant(s) in the center. The young plant is inserted though the disk and the hole is sealed around the root-neck with putty a few days after germination. These disks can also be adapted to holes in the base of the large chambers. Two to four small chambers can be placed in a large chamber unit, which gives light and supplementary air-conditioning. Air circulation is ensured by a fan or a Venturi pump.

Humidity is fixed by condensed water, and by measurement of water uptake in the case of hydroponic culture.

III - ANALYSIS AND QUANTITATIVE CONTROL OF ATMOSPHERES

ANALYSIS OF SHOOT AND ROOT ATMOSPHERES. Each chamber is linked by a network of pipes to the multichannel gas introduction systems of the CO_2 analyser and the mass spectrometer. Solenoid valves controled by a microprocessor draw up gas samples which expand either into the CO_2 analyser or into the mass spectrometer. One analysis lasts 15 seconds ; because of necessary vacuum periods, the CO_2 analyser is limited to 60 analyses per hour and the mass spectrometer from 30 to 40.

The analysis of a given chamber atmosphere is programmed to occur at given times, from 1 to 12 times per hour, according to the needs of the experiment. The infrared gas analyser (IRGA) takes 50 ml samples, which prevents its use with small volume chambers. The mass spectrometer uses only 0.5 ml or less gas for each analysis.

This system has the following advantages :

- it is simple : only one introductory valve per circuit for the spectrometer, one for the CO_2 analyser,
- sampling times can be freely programmed,
- unlike more traditional devices, it is not sensitive to the flow rate in the gas circuit,

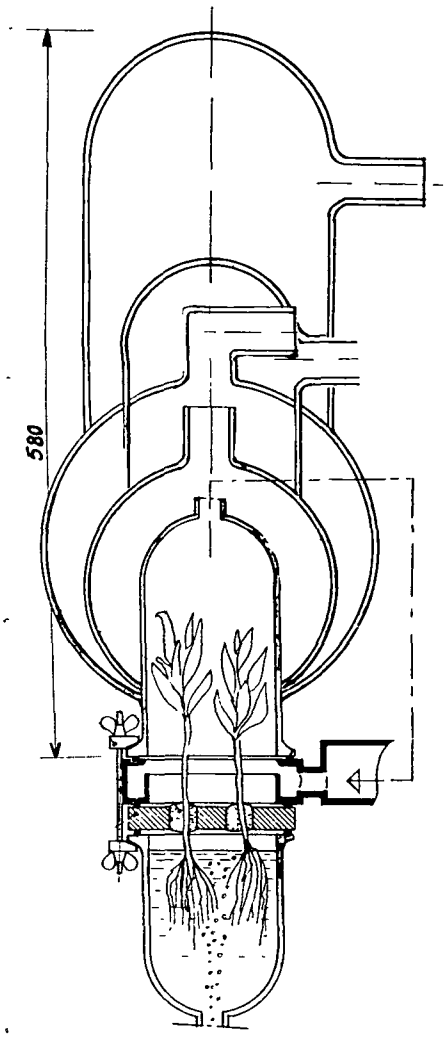


Figure 3 - Small chamber with different types of cloches of glass for shoot compartment. The module of air circulation and cooling is not shown.

- mixing of gas from different circuits never occurs. This is especially important when isotopes ($18O_2$, $14\ CO_2$) are used or when chemical products are tested.

But the main advantage is the quality and the safety of the measurements obtained :

- comparisons between chambers are always allowed, whatever the stability or calibration of the apparatus,
- calibration can be automatized. In the case of the mass spectrometer, this allows a tenfold increase of precision, especially with O_2. Each measurement is numerically compared with the measurement of a reference gas, so that the accuracy approaches that of differential measurements.

QUANTIFICATION OF THE REGULATION OF CO_2. The microprocessors associated with the chambers use the output signals of the centralized (for large chambers) or local (for small chambers) CO_2 analyser, and control injections of CO_2 during photosynthesis, or trapping of CO_2 during respiration, so as to maintain the CO_2 level at the programmed value. CO_2 injections are made by Solenoid valves which give calibrated quantities of CO_2 at each excitation. The number of injections measures the photosynthesis. The regulation adapts the frequency of pulses to the rate of photosynthesis so as to avoid any systematic lag between the real level of CO_2 and the set-point.

Data entered into the computer are the volume of the chamber and that of the injection of CO_2. The range of injection frequency is very large (1 to 100), allowing the measurement of photosynthesis of plantlets or adult plants without modifying any calibration. The regulation of the CO_2 level during respiration operates by opening or closing a CO_2 trap containing soda lime. The amount of CO_2 envolved by the plant, or trapped, is proportional to be duration of the opening of the trap, to the flow rate and to the concentration of CO_2, in the gas. The regulation program takes these experimental parameters into account to calculate the CO_2 exchange rate and to continue the regulation between times of measurement, which can be as far apart as 20 minutes.

THE MASS SPECTROMETER. The computerized control of the mass spectrometer allows repetitive analysis of a sequence of numerous atmospheres, including references gases.
This procedure ensures an exceptional accuracy of the results, although the quadrupolar mass spectrometer per se is not particularly accurate, but is does a lot of measurements rapidly for example of 120 measurements on 6 peaks in 12 seconds, the total duration of the sampling and measurements is 15 seconds. Measurements are taken only on the useful peaks of the spectrum concerning each atmosphere. Results are expressed as relative concentrations. The values given are the difference to a reference gas of reconstituted air.

This corrects the isotopic concentrations from the natural or artifactual background, and corrects the CO_2 concentrations from the background due to the oxidation of the filament. The hourly repetition of the sampling sequence and the adequate programming of the sampling order in the sequence prevent disturbance by memory effects, even at a high frequency of analysis. An example of the performance of the mass spectrometer over 3 days and of its interest in the study of root respiration is shown in Figure 4.

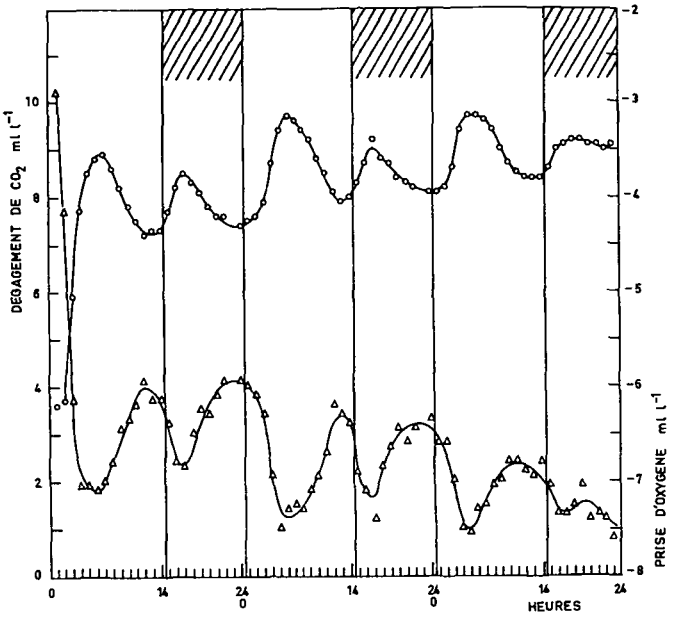


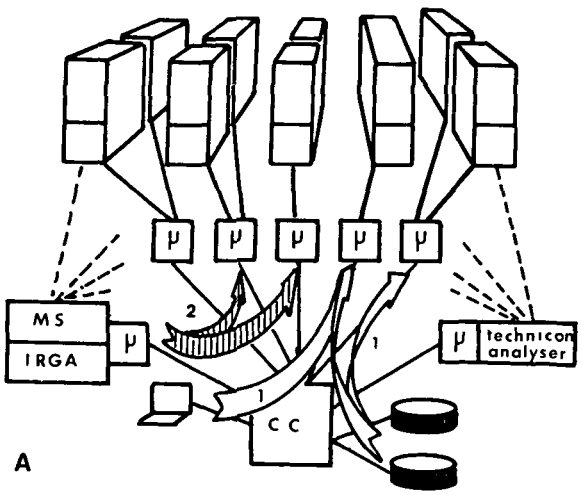
Figure 4 : Rate of root respiration of wheat plant measured by mass spectrometry (M.S) (o) CO_2 evolution, (Δ) O_2 uptake. The root container was aerated by CO_2 free air. The M.S. measured the change of CO_2 and O_2 concentrations due to the root metabolism.

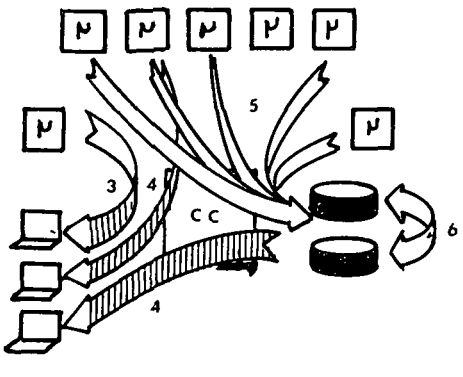
The O_2 uptake was obtained by the difference of O_2 concentration between the entrance and the exit of root system i.e. by the fullscale measurement of about 1.10^{-2} on the background of air, $20.6\ 10^{-2}$.

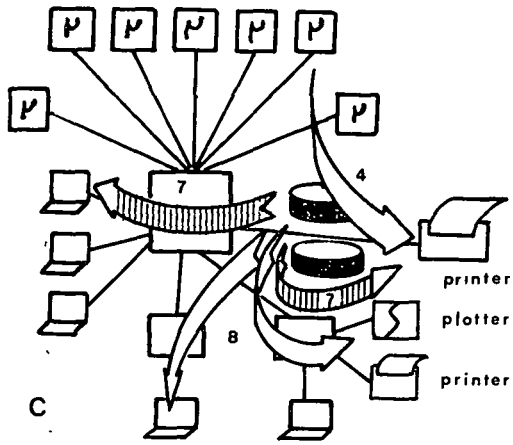
IV - AUTOMATIC CHEMICAL ANALYSIS

The system used (Technicon autoanalyser) is well-known in the field of analysis by colorimetry. It was an original application to associate it with a system of micro-electrovalves and catheteral sampling. The chemical analyzers are also controlled by a specific microprocessor, which commands frequent calibrations with reference solutions, and the accuracy of analysis is always better than 0.5 %. Using experimental parameters (volume of the containers, initial concentrations, etc...) the computer directly calculates uptake rates for each element generally, NH_3, NO3, P, and K but also Ca, S, Mg, Mn and Cl if necessary.

Real time use of the automatic chemical analysis is limited (Massimino et al, 1981), the most frequent use being the delayed analysis of nutrient solution samples that are taken everyday and stored at - 18°C. Then series of samples covering a whole experiment /for example the whole life cycle of a Maize crop (André et al, 1978 b) or the vegetative stage of wheat Ducloux, 1984/ are analysed in one run and daily uptake rates can be calculated as above and filed on disks.







COMPUTEUR SATELLITES

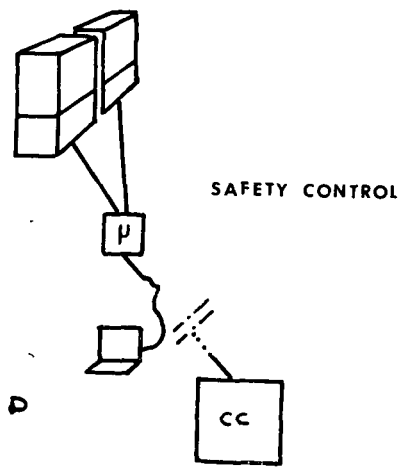


Figure 5 : Functions of the central computer (CC) connected with :
A) The micro-processors (μ) for the control of the growth chambers, the IRGA-mass spectrometer system and the Technicon Autoanalyzer.
B) Two discs as data base.
C) The mini-computers satellites for modeling and tele-processing.
D) Safety connection during maintenance or disturbance of central computer.

N°	Function	Command by	Exchanges from to
1	Loading of	Display consol I	Discs, display μ
2	Exchanges of data	Clocks and soft	μ ⟶ μ
3	Control of process	Display & consols	⟶ Displays & printer
4	Control of data	Display & consols	μ , discs ⟶ Display & printer
5	Storage of data averages	Clock & soft	μ ⟶ Discs
6	Duplication files processing	Soft-Keyboard I	Disc ⟶ Disc
7	Standard calculation	Display consols	Discs ⟶ Display printer
8	Data transfer	Satellites Computers	Discs ⟶ Satellites

V - THE COMPUTER SYSTEM

Its architecture is organized around a central data base in a computer linked to decentralized autonomous terminals :

- Seven microprocessors controling the culture chambers and measuring systems. They attend to the acquisition of measurements of the physiological activity (photosynthesis, respiration, photorespiration, transpiration, root respiration, nutrition) and of environmental parameters.
- Peripherals for data control (visual display terminals, printer).
- Terminals for data processing, calculating, modeling and programming.

THE MICROPROCESSOR. It uses a Motorola 6809 associated with a G64 bus and industrial peripherals ("Europe" card) developed by GESPAC (CH R28 Genève, Switzerland) and Thomson-EFCIS (F 78140 Velizy-VillaCoublay, France).

Its characteristics are a memory of 32 K bytes, 16 analogical inputs, 8 temperature imputs (Pt 100 sensors) 4 analogical outputs, 64 channels of logical imput-outputs.

The control and regulation software is configurable with 30 modules of elementary functions (sequence, comparison threshold, proportional regulation, integration, derivate, input, output, etc...).

One microprocessor can control two chambers. The gas analysis system (IRGA and mass spectrometer) and the Technicon chemical analyzer are controlled by two specific microprocessors, optical fibers connect the central computer and the micro-processors. The general software was studied by the CISI company (F13115 St Paul-Lez-Durance)

THE CENTRAL COMPUTER. We chose an industrial computer the Solar 16-40 produced by Bull-SEMS (Echirolle 38000 France) because a former one had proved very reliable. Its main features are the following :

- memory of 256 K-Words of 16 bits,
- communication processor (IOP) and floating point processor (FPP),
- RTDES real time disk operating systems.

It is associated with two disk units of 10 M-bytes, each with a fixed head disk and a removable disk for data archival. (Figure 5)

THE SATELLITES MICROCOMPUTERS. (Bull-micral 30, compatible IBM PC) They use disk archival files and are programmable in BASIC for non routine data treatment. Programs written on the satellite terminals can be tranferred to be used in the central computer in compiled BASIC. The long distance transfer of the data has been envisioned as part of a European project.

SECURITY. Our experience of using a computer for the direct control of experiments, since 1976, has shown that the computer was the most reliable part in the whole experiment, with a time availability ratio of over 99 %.

This is made possible by the quality of the hardware but also by the safety procedures used, as preventive maintenance, self-testing and diagnosis of the computer. The new descentralized system should be as reliable, thanks to the following precautions :

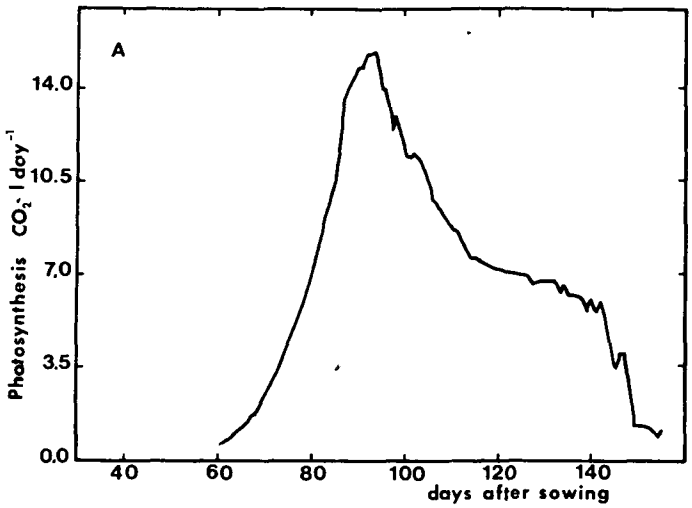
- In case of a default in the central system, the microprocessors associated with the chambers can control the experiments and store the data within three hours.
- They can be directly connected with the control display terminals and a simplified management ot the experiments is possible in case of a lengthy deficiency of the central computer.
- Back up of the software and data is ensured by a systematic copy procedure. The two disks are used symmetrically copy procedure. The two disks are used symmetrically so that one can replace the other.

VI - EXAMPLES OF RESULTS

The leading idea of our experiments was to study the contribution of the main photo-phenomena : photosynthesis and photorespiration, to the energy budget of the plants. We also looked at dark respiration of shoots and roots and even root excretion in some particular studies. These balances were studied at the scale of a plant life cycle, or in a vegetative stage in light of environmental parameters. The parameters (light, CO_2, O_2, water) were chosen to change the value or the proportion of the two photo-phenomena. In a whole plant approach the interactions with other physiological activities were considered to uncover a possible role of the photorespiration.

LARGE CHAMBERS. They have first been used for the simultaneous study of shoot and root physiological activities, either during the life cycle of a Maize plant (8,9) or under the effect of a light reduction simulating a cloudy day (11,12). Data files were used later for the adjustment of growth and maintenance respiration models (fig. 6) (13) (14).

Maize is a plant with a very low photo-respiration, only the photosynthesis was concerned, but these studies can be used as base line for experiments manipulating both photosynthesis and photorespiration.



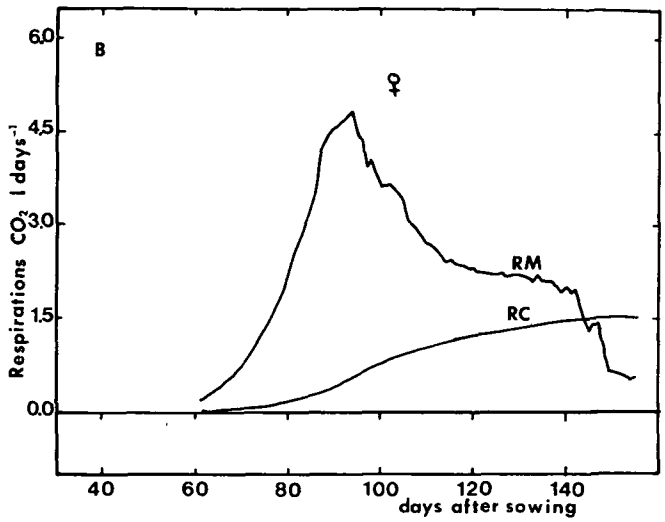


Figure 6 : Paterns of A) Photosynthesis (P) and B) Respiration (R) during the life cycle of maize crop. The measured respiration was shared to the sum of two terms : growth respiration Rc = αP, and respiration of maintenance R_m = bΣ (P - R) following the assumption :
R_t = aP + bΣ (P-R) (adjustment by last square method a = 0.27, b = 0.0032). Rm was very low in the vegetative stage (♀ = silking).

In the case of the wheat plant the acceleration of growth at an increased level of CO_2 is due to both the stimulation of photosynthesis and the inhibition of photorespiration (Fig. 7)

Curves are drawn from data files, without correction or smoothing. The ratio between the two curves is the growth stimulation coefficient. It remains stable throughout the experiment. Contrary to previous studies (15) we do not observe any negative feed-back effect from the enhancement of photosynthesis. The results are part of a more comprehensive study of the effect of CO_2 on photosynthesis, photorespiration, transpiration, nutrition and draught effects (Du Cloux, 1984).

SMALL CHAMBERS

They are adapted to the study of photorespiration with ^{18}O. The mass spectrometry technique allows the continuous measurement of O_2 uptake and evolution during photosynthesis. The first studies in wheat under standard growth conditions, showed that O_2 uptake during photosynthesis was nearly as fast as net photosynthesis (16, 17), which corresponds to a loss of reductive energy of 50 %. This is two times as much as what could be predicted from the biochemical analyses in-vitro of the properties of RuBP carboxylase-oxygenase (18). Research is in progress for understanding the reasons for this difference and possibly finding a physiological role for photorespiration. The current hypothesis of a protective role is supported by the finding that in case of water stress, in the soybean, two-thirds of the reductive power is diverted to photorespiration (19). Also, a study of gas

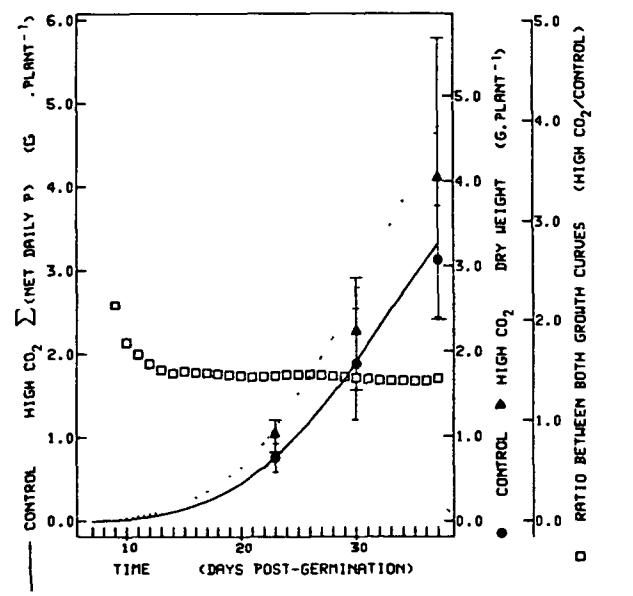


Figure 7 : Growth of a canopy of wheat (Triticum aestivum L. var. Capitole) during the vegetative phase at normal (—) or double (---) concentration of CO_2. Dry weight was calculated by the cumulation of daily CO_2 exchange balance, on the assumption that 1 g dry weight corresponds to 440 mg carbon, stimulation coefficient (□) weights measured on randomly sampled and sacrificed plants (10 plants per sample) (●, ▲). Standard deviation bars show the large dispersion of results obtained by sampling compared with the stability of gas exchange measurement ratios. Plant density was 200 m^{-2}, PAR 600 µmol photons m^{-2} s^{-2}, temperature 24/18°C, photoperiod 14/10 h, R.H. 50/85 %.

Exchanges in mosses showed that the maximum oxygen uptake capacity is low in these primitive plants. We suggest that evolution towards a greater capacity of oxygen uptake is a necessary condition to support stress resistance in higher plants.

Small chambers are also well adapted to the study of shoot-root relations, which are a key to the understanding of whole plant physiology (Figure 4 above).

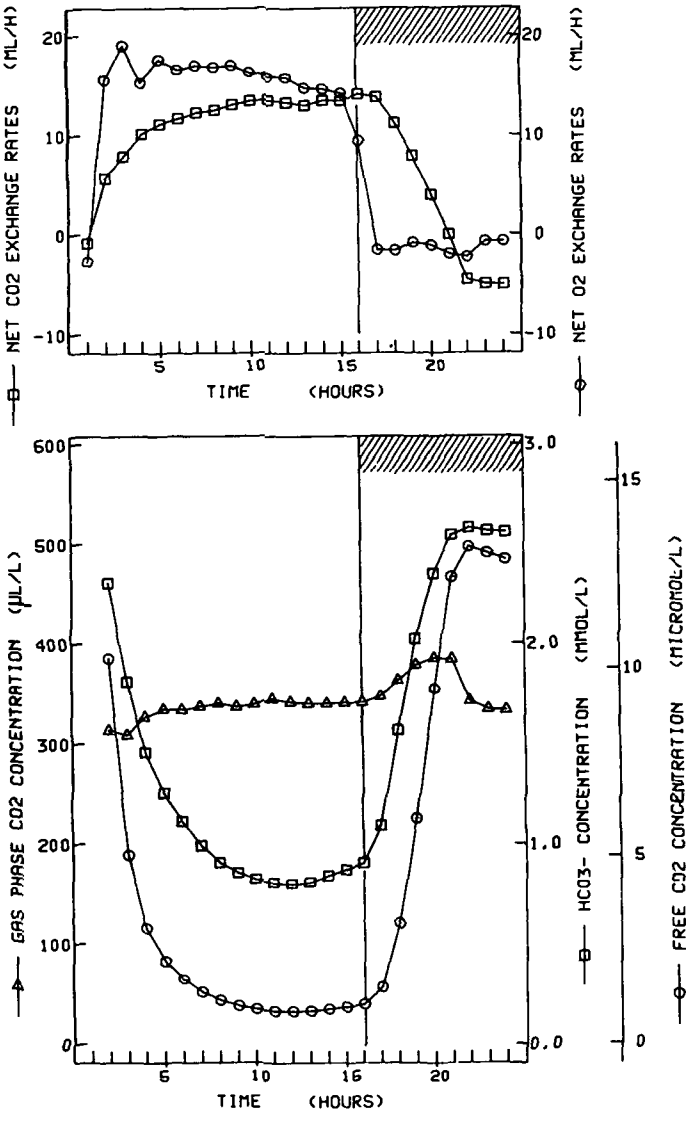


Figure 8 : Photosynthesis in the marine macroalga Chondrus crispus. A) daily cycle of the gas exchanges as measured in the air or in the reactor : (O) O_2 production or uptake, (■) apparent CO_2 uptake (as measured by the regulation)
B) Variations of the various forms of inorganic carbon in the system : (○) free dissolved CO_2, (□) bicarbonate ions, (△) gas phase CO_2 concentration.

REACTORS FOR AQUATIC PLANTS. The study of O_2 and CO_2 uptake in Chondrus crispus has shown that the oxygenation is slow in normal conditions and that it is due only in part to the glycolate pathway. It was shown that, like certain microalgae, bicarbonate can be taken up directly (21,22) and even preferentially to CO_2 (23). Figure 8 shows an example of curves obtained by computer.

The reactor contains 4 liters of sea water in dynamic equilibrium with 1 liter of air, 30 g of algae (fresh weight). PAR : 200 mol photons m^{-2} s^{-1}. The atmosphere was recycled through the water with a flow rate of 120 l h^{-1}.

VII - CONCLUSION

The system described has been shown to advance research in the study of whole plants, compared to previous equipment, thanks to the following advantages :

- It can simulate a real climate or create an artificial one, with the possibility of modifying one parameter or the composition of the atmosphere at any time.
- Cultivation can be prolonged indefinitely in these conditions, while measurements are automatically and continuously taken and stored.
- The large number of parameters measured, and their accuracy, give at every moment a relatively complete picture of the plant activity and of its environnent.
- Mass spectrometry allows the measurement and study of photorespiration, an important component of the gas exchanges (the flow of O_2 is four times that of the dark respiration). The use of other isotopes (^{15}N, ^{13}C, ^{14}C) remains possible.

The price paid for these results is moderate. The initial investment (mainly in the computer system) has been amortized in more than ten years, so that the annual cost of the computer was less than the salary of two gardeners.

The field of possible applications is vast. Long-term as well as short-term effects of any treatment (increase of the level of CO_2, climatic variation, disease, nutrient deficiency, growth regulations, herbicides etc...) can be studied in unequaled conditions of precision. Automatization allows the realization of these experiments with the minimum of staffs : two persons can easily conduct one double experiment in twin chambers.

The capacity of our centralised equipment allows for more possibilities that our limited team, focussed on photorespiration, can use, and gives a possibility for collaboration with other laboratories.

ACKNOWLEDGEMENTS

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Description of Concept and First Feasibility Test Results of a Life Support Subsystem of the BOTANY FACILITY Based on Water Reclamation

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ABSTRACT:

The BOTANY FACILITY allows the growth of higher plants and fungi over a period of 6 months maximum. It is a payload planned for the second flight of the EURECA platform around 1990.

Major tasks of the Life Support Subsystem (LSS) of the BOTANY FACILITY include the control of the pressure and composition of the atmosphere within the plant/fungi growth chambers, control of the temperature and humidity of the air and the regulation of the soil water content within specified limits.

Previous studies have shown that various LSS concepts are feasible ranging from heavy, simple and cheap to light, complex and expensive solutions. In the first part of the paper a summary of those concepts is given. In the second part a new approach to accomplish control of the temperature and humidity of the air within the growth chambers is described which is based on water reclamation. This reclamation is achieved by condensation with a heat pump and capillary transport of the condensate back into the soil of the individual growth chamber.

Part three provides some analytical estimates in order to obtain guidelines for circulation flow rates and to determine the specific power consumption.

The design of a water reclamation module is described in part four while the test hardware is illustrated in part five. Part six describes the test set-up while in the seventh and last part of the paper the test results are summarized and discussed.

1. INTRODUCTION

One of the core experiments of the second flight of EURECA (= EUropean REtrievable CArrier) currently scheduled for 1990 is the BOTANY FACILITY (BF) which is a plant growth chamber designed to accommodate various experiments with plants and/or fungi. Table 1 summarizes the BF performance data with the emphasis laid on the functions the life support subsystem (LSS) has to fulfill.

Purpose	:	Growth of higher plants and fungi from seed to seed and spore to spore
Carrier	:	EURECA
Mission duration	:	6 months maximum
Features	:	• 12 micro gravity cuvettes • 6 1g control cuvettes • Illumination • Video • Data acquisition • Pollination device • Fixation device
Life support subsystem functions	:	• Contamination control • Air humidity control • Control of soil water content • Control of atmospheric pressure • Control of composition of atmosphere
Developmental status	:	• Phase B study completed • 1. flight on 2. EURECA mission
BOTANY FACILITY performance data		

Table 1: BOTANY FACILITY performance data

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An impression of the BF geometry existing at the end of the phase A study can be gained from Fig. 1.

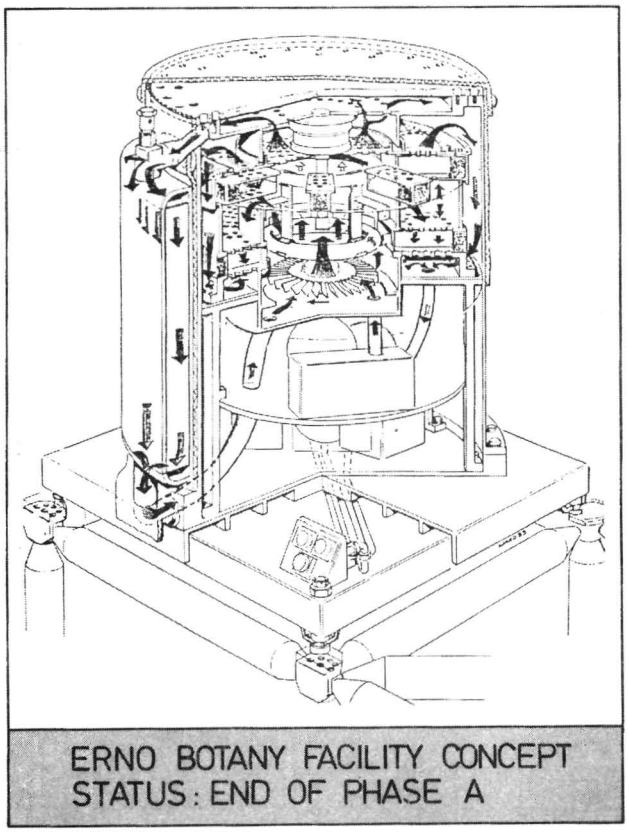

Fig. 1: ERNO BOTANY FACILITY CONCEPT STATUS: END OF PHASE A

Early in the study it turned out that the LSS conveniently should be divided into a Ventilation And Soil/Air Humidification (VASAH) loop and an Atmosphere Storage And Composition Control (ASACC) loop. For each of them a number of options have been described and discussed in a previous paper, see (1)*.
For each set of options a trade-off was conducted considering aspects such as

- o Weight
- o Technical complexity
- o Critical components
- o Compatibility with microgravity environment
- o Costs

resulting in the recommended options (status end of phase A) which are depicted in Fig. 2 and 3.

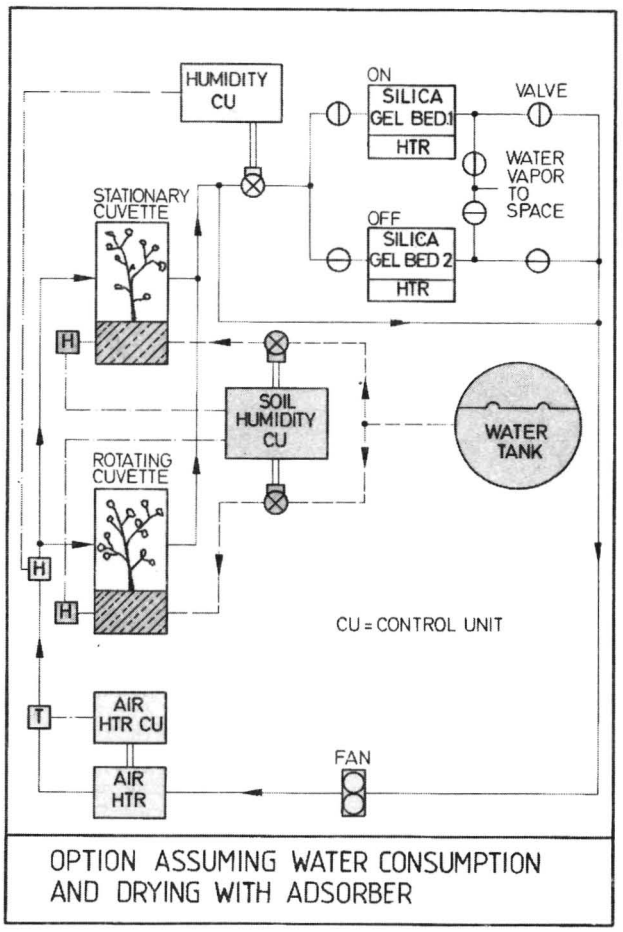

Fig. 2: VASAH loop option based on water adsorption in a regenerable Silicagel dryer

* Numbers in parenthesis designate references at end of paper

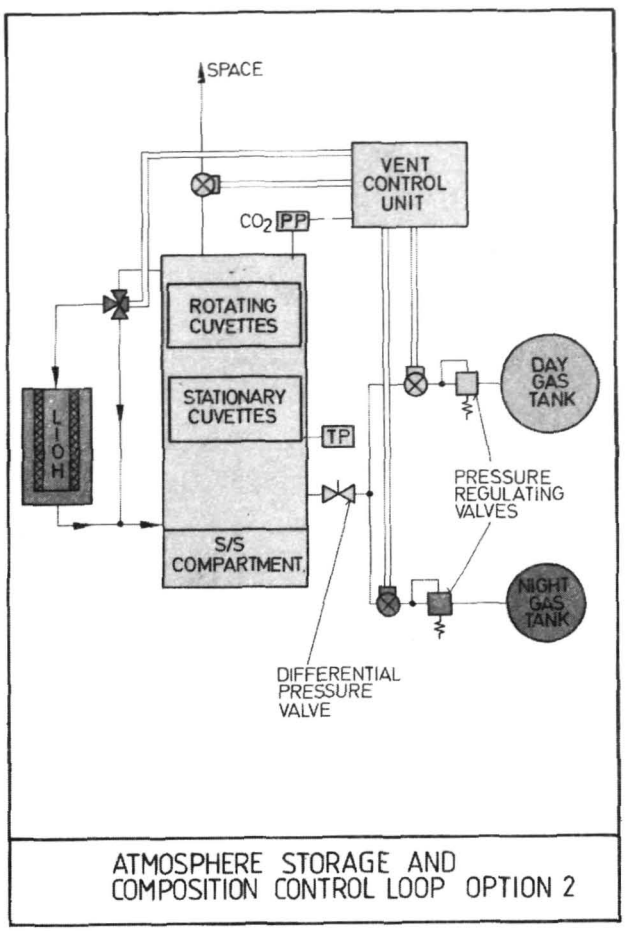

Fig. 3: ASACC loop option using day and night gas tank in combination with Lithiumhydroxide cartridge

As can be seen from Fig. 2 water is stored in a water bladder tank and is at the begin of the growth period allowed to moisten the soil by means of the soil humidity control unit (which can be part of the on-board data handling system). For that purpose soil humidity sensors are used which cause the watering valve of a particular cuvette to close in case the desired soil water content has been reached. Obviously, each cuvette needs its own sensor because different plants or plant sizes might cause different water consumption/resupply.
The water evaporated by the surface of the soil or a solution containing nutrients (AGAR) and the plant is carried away by the air circulating through the cuvettes and an air humidity sensor controls the position of a bypass valve allowing a certain amount of air to flow through the active Silicagel bed. Active designates the bed which is not regenerated. By adsorption the Silicagel removes a certain fraction of the water contained in the air with the result that the air leaving the bed is very dry. By mixing that air flow with the bypassed flow the desired humidity at the inlet of the cuvettes is achieved. The air returning to the cuvettes passes through a fan which provides the necessary differential pressure to overcome the pressure loss of the various loop components.

In order to achieve a close temperature control the air passes through a heater before it returns to the cuvette.
To avoid cross-contamination of the various cuvettes at the inlet and the outlet of each cuvette a sterile filter is positioned (not shown in Fig. 2).

After a certain time interval the adsorption capacity of a dryer bed is reached and it will be isolated from the loop by means of solenoid valves. The second bed - which in the meantime has been regenerated by the combined effect of space vacuum and elevated temperature - is connected with the loop and takes the function of the first bed.
The function of the ASACC loop is discussed in detail in (1) and shall not be addressed here.

2. CONCEPT OF WATER RECLAMATION

As will be noticed, the VASAH concept illustrated in Fig. 2 is based on water consumption which means that all the water needed during a mission has to be considered as a consumable.
Apart from the resulting weight penalty the concept is fairly complex, in particular the water supply to the rotating cuvettes located in the control centrifuge will not be an easy task.
Therefore the concept based on water reclamation was re-evaluated which had been addressed already in (1) but was rejected due to its apparent complexity.
Fig. 4 illustrates in a schematic form the concept of internal water management.

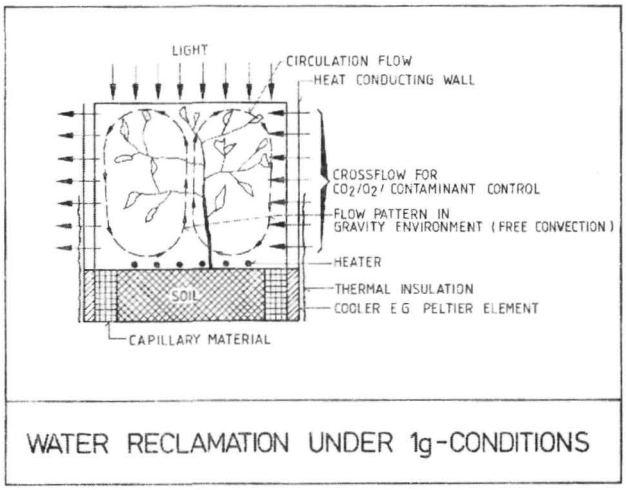

Fig. 4: Cuvette with closed water loop feasible for 1 g-environment

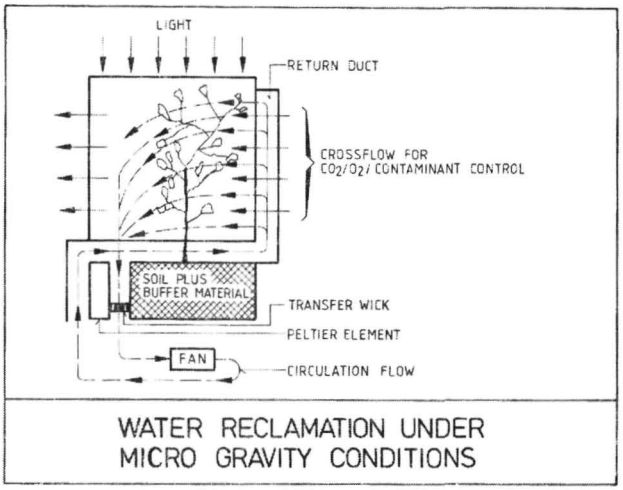

Fig. 5: Cuvette with closed water loop feasible for microgravity environment

As can be seen, the lower portion of the cuvette sidewalls is manufactured from a material having a good thermal conductivity and is connected with a cooler, e.g. a Peltier element. The cooler in turn is in contact with the soil by means of a capillary material. Atop of the soil several heater wires are installed, however, tests may prove that they are not required.

The light input and - if required - the heat liberated by the heater wires will warm up the air in the center position of the cuvettes and it will rise due to the density difference. At the cooled sidewalls the opposite effect will establish and as a result a circulation pattern should prevail as illustrated in Fig. 4.
When the air is progressively cooled along the sidewalls, condensation will occur and the water droplets formed will dripple into the capillary material (wick) which allows the water to return to the soil.
The air flowing across the soil surface and passing the heater wires is comparatively dry such that the plant (and the soil surface) will evaporate water by taking it from the soil.
As can be seen, a closed water loop with two phase changes should form: In the plant's leaves from liquid to vapour state and at the condenser from vapour to liquid.
The air humidity in the cuvette can be controlled for a given plant size by varying the power applied to the Peltier element.

Under microgravity conditions the air flow pattern caused by density differences disappears and one has therefore to replace free by forced convection by installing a fan. A conceivable arrangement is shown in Fig. 5.

When compared to Fig. 4 there are 7 modifications depicted in Fig. 5:

1. A ducting system for the supply of dry air to the cuvette and the return of wet air to the condenser.
2. Installation of a mini fan to overcome the pressure loss of the ducting system.
3. Deletion of heater wires.
4. Thermal decoupling of sidewalls from the condenser.
5. Modification of the condenser such that a good thermal contact exists between its surface and the air flowing past it.
6. Introduction of a transfer wick which allows the transport of the condensate from the condenser to the soil.
7. Installation of regenerative heat exchanger to reheat the air leaving the condenser to cuvette temperature by using a portion of the waste heat of the Peltier element (not shown in Fig. 5).

The major difference relative to the concept feasible under 1 g-conditions is the use of capillary forces in order to separate the condensate from the air in the concept for microgravity conditions.

If one compares the concept of Fig. 5 with the option 4 depicted in Fig 8 of (1) one will recognize in the condenser of Fig. 5 the condensing heat exchanger of Fig. 8 of (1) and in the transfer wick of Fig. 5 the water separator of Fig. 8 of (1).

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Apparently a number of flow patterns are possible for the microgravity cuvette as shown in Fig. 5. For example the kinds illustrated in Fig. 6a-c can be imagined.

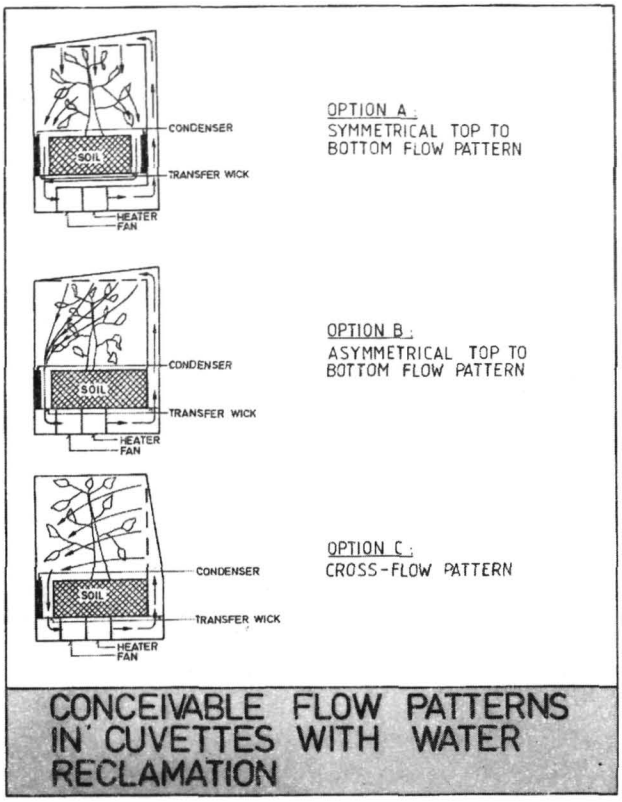

Fig. 6: Conceivable flow patterns for a microgravity cuvette with water reclamation

The most sophisticated pattern is that shown in Fig. 6a. Since it is symmetrical it requires 2 condensers and 2 transfer wicks. Another disadvantage is seen in the fact that the top surface of the cuvette is used to distribute the air supplied to the cuvette. Normally one would want to reserve that zone as a light entrance area. Fig. 6b shows a simplification of the previous option in that only one condenser/transfer wick is foreseen and therefore the flow pattern will be asymmetrical. Fig. 6c finally shows the option being apparently the simplest: Asymmetrical cross flow. Note that the top surface (lid) of the cuvette is available for undisturbed light entry.

A compilation of the advantages and the disadvantages of the water reclamation concept is given in Table 2.

ADVANTAGES	DISADVANTAGES
1. Rotating (1g) cuvettes need no water supply.	1. Each cuvette needs its own instrumentation.
2. Imbalance problem greatly relieved due to item 1.	2. Each cuvette needs its own Peltier cooler.
3. Neither the 1g- nor the micro-gravity cuvettes need a soil humidity sensor.	3. Each micro-gravity cuvette needs its own K/S-fan.
4. Water no longer a consumable.	4. If a variation of the soil water content during the growth period is desired, a small dryer is needed.
5. Subsystem hardware weight reduced due to absence of Silica gel dryer.	5. Power consumption of Peltier elements and heater.

ADVANTAGES AND DISADVANTAGES OF CUVETTES WITH WATER RECLAMATION

Table 2: Advantages and disadvantages of cuvettes with water reclamation

3. ANALYSIS OF WATER RECLAMATION CONCEPT

Fig. 6 illustrates the loop schematic as far as thermal and electrical aspects are concerned. At first, a water balance shall be made. For the cuvette this balance reads:

$$\dot{G}_A \cdot (x_o - x_i) = \dot{G}_W \qquad (1)$$

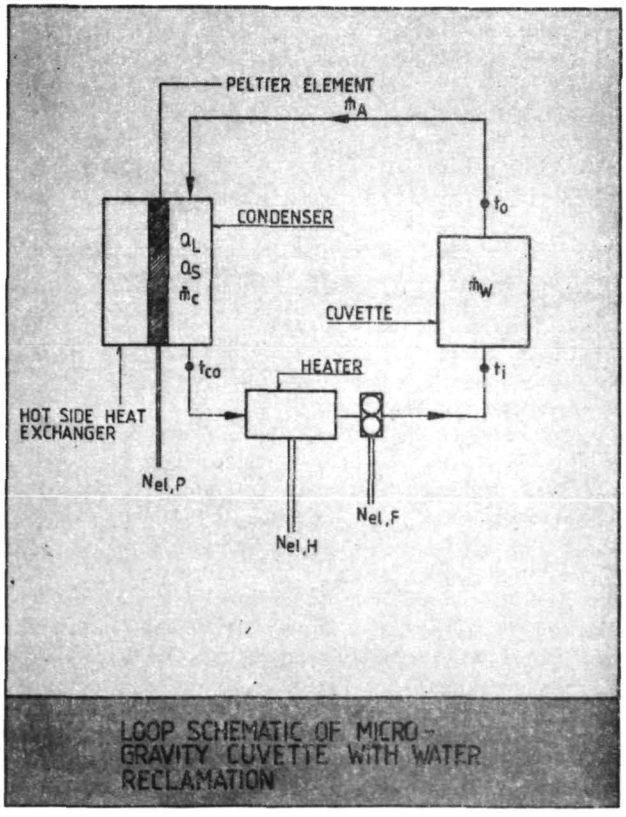

Fig. 6: Loop Schematic of microgravity cuvette with water reclamation

Similarly, the water balance for the condenser can be written as

$$\dot{G}_A \cdot (x_o - x_{Co}) = \dot{G}_C \qquad (2)$$

since

$$\dot{G}_W = \dot{G}_C \qquad (3)$$

and because one will normally prescribe x_o, one obtains from eq. (1), (2), (3) the following relation for x_{Co}

$$x_{Co} = x_o - \dot{G}_W/\dot{G}_A \qquad (4)$$

From the relation between water content, x_{Co}, and air temperature, the air temperature at the condenser outlet, t_{Co}, can be determined. As can be seen from eq. (4), t_{Co} is a function of the cuvette air temperature and relative humidity and of the ratio $\dot{G}_W/\dot{G}_A$. Fig. 7 illustrates the variation of t_{Co} with $\dot{G}_W/\dot{G}_A$ for a cuvette air temperature of 20 °C (68 °F) and a relative humidity of 80 % leading to x_o = 12.15 g/kg.

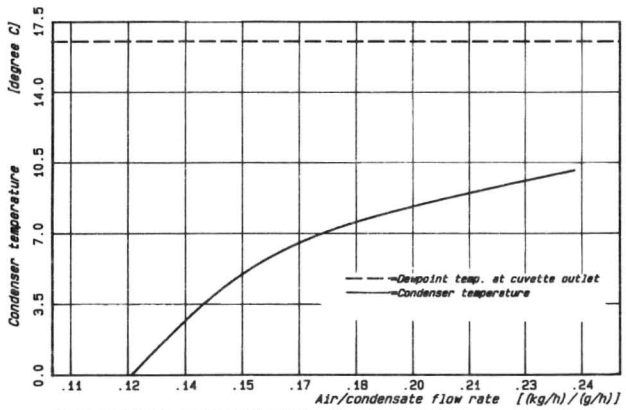

Fig. 7: Temperature at the condenser outlet as function of the air mass flow rate, $\dot{G}_A$, divided by the condensate flow rate, $\dot{G}_W$

Two limits are apparent in Fig. 7: Minimum $\dot{G}_A/\dot{G}_W$ ratio is 0.127 (kg/h)/(g/h) because here the freezing point is reached. Obviously, one must not operate under that condition because no water transport is possible at that temperature. An infinite air flow rate is needed on the other hand if one wants to remove the evaporation rate $\dot{G}_W$ under the condition that the air temperature at the condenser outlet is equal to that at the cuvette outlet, this characterises the second limit.

The next step is now to formulate a heat balance. Total condenser load is

$$\dot{Q}_C = \dot{Q}_s + \dot{Q}_l \qquad (5)$$

Sensible load

$$\dot{Q}_s = c_p \cdot \dot{G}_A \cdot (t_o - t_{Co}) \qquad (6)$$

Latent load

$$\dot{Q}_l = r \cdot \dot{G}_W \qquad (7)$$

Combination of eq. (5) (6) (7) yields

$$\dot{Q}_C/\dot{G}_W = c_p \cdot \frac{\dot{G}_A}{\dot{G}_W} \cdot (t_o - t_{Co}) + r \qquad (8)$$

For the heater power to heat the air reentering the cuvette (see Fig. 6) one obtains after normalization with $\dot{G}_W$

$$\dot{Q}_H/\dot{G}_W = c_p \cdot \frac{\dot{G}_A}{\dot{G}_W} \cdot (t_i - t_{Co}) \qquad (9)$$

Obviously, $t_o = t_i$.

Fig. 8 shows an numerical evaluation of eq. (8) and (9) as function of $\dot{G}_A/\dot{G}_W$ for an air temperature of 20 °C and a relative humidity of 80 %.

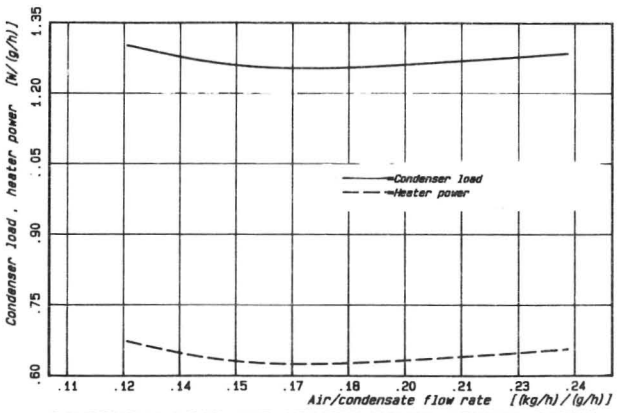

Fig. 8: Normalized condenser load $\dot{Q}_C/\dot{G}_W$ and heater power $\dot{Q}_H/\dot{G}_W$ as function of ratio $\dot{G}_A/\dot{G}_W$.

As can be seen from Fig. 8 an optimum value for $\dot{G}_A/\dot{G}_W$ exists for which both the condenser load (and hence the load to be removed by the Peltier element) and the heater power attain

a minimum. This optimum is explainable by the fact that at the lower limit of Fig. 7 the temperature difference in eq. (8) and (9) (in fact the sensible heat load) reaches a maximum and the flow rate $\dot{m}_A$ a minimum while at the upper limit of Fig. 7 the opposite is true.

The optimum ratio corresponds to

$$(\dot{G}_A/\dot{G}_W)_{opt} = 0.19$$

and the corresponding temperature of the air at the condenser outlet is $t_{Co} = 8.1$ °C.

To get a feeling for the absolute power values it shall be assumed that $\dot{G}_W = 0.1$ g/hr. Then follows $\dot{Q}_C = 0.13$ W and $\dot{Q}_H = 0.06$ W for the optimum mass flow ratio $(\dot{G}_A/\dot{G}_W)_{opt}$.

Finally, the required electrical power to operate the Peltier element shall be estimated. As will be recalled, a measure of the quality and the actual operation condition of a Peltier element is the Coefficient Of Performance (COP) which is defined as

$$COP = \frac{\text{heat absorbed by cold side}}{\text{electrical input power}} \qquad (10)$$

The heat to be removed on the hot side of the element is

$$\dot{Q}_{P,H} = N_{el,P} + \dot{Q}_C \qquad (11)$$

Combination of eq. (10) (11) results in

$$\dot{Q}_{P,H}/N_{el,P} = 1 + COP \qquad (12)$$

Typical COP-values are in the neighbourhood of 0.3. This then leads for the evaporation ratio used in the example, namely $\dot{G}_W = 0.1$ g/hr to $N_{el,P} = 0.43$ W and to $\dot{Q}_{P,H} = 0.56$ W.

By using a regenerative heat exchanger a portion of the hot side heat load of the Peltier element can be used to reheat the air returning to the cuvette and therefore the heater power $N_{el,H}$ (0.06 W in the example) can be saved.
Power for the mini-fan is according to current experience appr. 0.2 W. So, assuming zero heat leaks and heat regeneration the total required electrical power would be 0.43 + 0.2 = 0.63 W for an evaporation rate of 0.1 g/hr.
Presently 18 stationary (microgravity) and 6 rotating (1 g) cuvettes are specified in the BF requirements. Neglecting the fact that the heater needed in the 1 g-cuvettes very probably will have a lower dissipation than the fans in the micro-gravity cuvettes one will end up with a total electrical power consumption of appr. $N_{el,total} = 24$ W assuming zero heat leaks.
Compared with the budget allocated to the BF of 160 W this is already a significant although not unacceptable amount.

So the practical design must be carefully optimized to minimize heat leaks in order to come at least close to the theoretical performance of $N_{el,\ P+F}/\dot{G}_W \cong 6.3$ W/(g/hr).

4. DESIGN OF A WATER RECLAMATION MODULE

In order to demonstrate the feasibility of the concept of cuvettes with internal water reclamation, a Water Reclamation Module (WRM) has been conceived, designed, manufactured and tested.
Since it was felt that the concept would be more difficult to verify for the microgravity cuvettes the design of the WRM was limited to that part.

As will be clear from the concept description, the critical element in a microgravity cuvette is the Condenser/Wick Assembly (CWA) which interfaces with the Peltier element.

The major requirements the CWA has to meet are as follows:

1. Large specific surface area to minimize temperature difference between Peltier element cold side and condenser surface with which the air comes into contact.

2. Wick material must have a good thermal conductivity for the reason mentioned in item 1.

3. Wick material must have a good capillary action.

4. Wick design must be self-priming, i.e. it must show capillary action even if it is initially completely dry.

Usual cotton wicks, for example, as used in commercial oil lamps have been considered in the beginning since they exhibit good capillary force but there are 2 drawbacks:

(a) They contain a small amount of cotton oil prohibiting water adsorption and
(b) thermal conductivity of cotton is very poor. The next thought was to use a wick made out of a grid of stainless steel. Two samples are shown in Fig. 9.

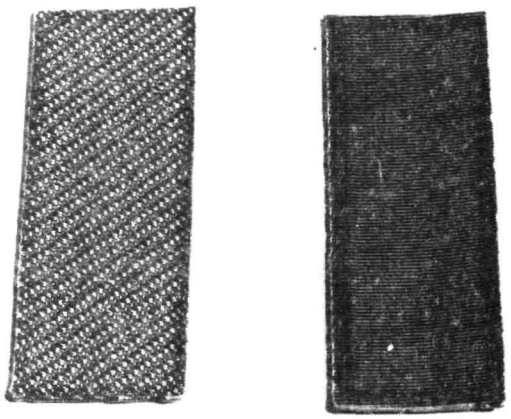

Fig. 9: Grid samples consisting of stainless steel wires. Edges are seamed by electron beam welding, length 70 mm, width 10 mm

Although the thermal conductivity was much better than that of a cotton wick, the self-priming requirement could only be met by adding a chemical wetting fluid to the water. Since we were not sure if that fluid would stay during a mission time of several months in the soil without degradation (and thus creating contaminants for the plant samples) also that wick material was rejected.

The following step was to think about a composite wick design, namely to use one material having a good thermal conductivity in order to fulfill requirements 1 and 2 and use a second material providing the capillary force and being self-priming to meet requirements no. 3 and 4. Therefore various copper nets have been evaluated as condenser element, 2 samples are shown in Fig. 10.

Fig. 10: Samples of copper grid material used as condenser material

As a wick material a commercially available capillary mat was used and the resulting design concept is shown in Fig. 11.

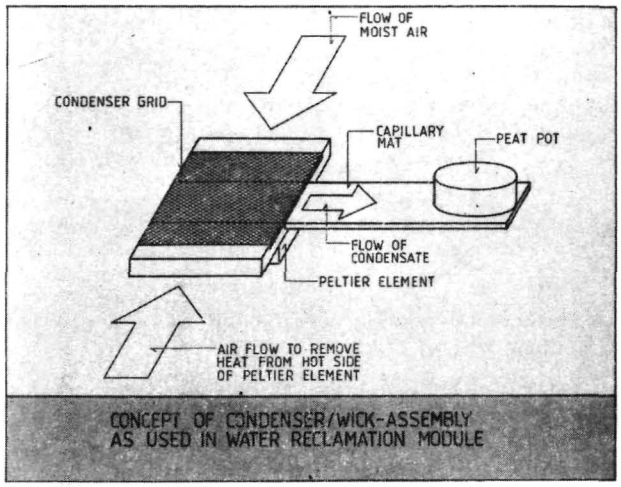

Fig. 11: Design concept as used for water reclamation module

The actual design is then illustrated in greater detail in Fig. 12.

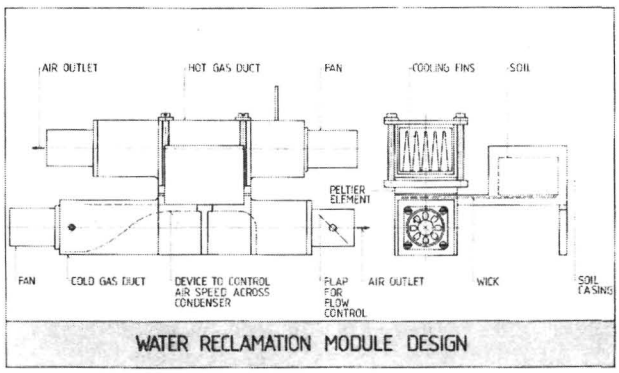

Fig. 12: Water reclamation module design

As can be seen from Fig. 12 ambient air is sucked by means of a small fan through the cold gas duct and is cooled/dryed at the condenser. The water absorbed by the wick assembly is transported to the soil which is contained in a casing in order to prevent evaporation.
The waste heat is removed by air drawn by another fan through the hot gas duct.

5. TEST HARDWARE

The actual hardware used for the tests is shown in Fig. 13 which depicts the WRM with thermal insulation in order to minimize heat exchange with the environment and from the hot gas to the cold gas duct. Insulation consisted of a foam material (ROHACELL) and a layer of goldized KAPTON.

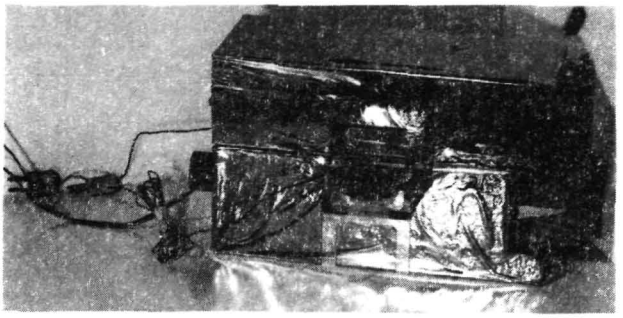

Fig. 13: Assembled WRM with insulation

Fig. 14 gives a better view how the thermal interaction between the 2 gas ducts other than via the cooling fans was tried to minimize.

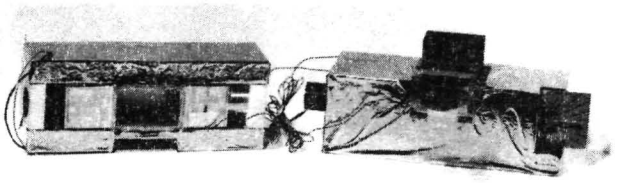

Fig. 14: Thermal insulation of cold and hot side air ducts

The disassembled WRM is shown in Fig. 15.

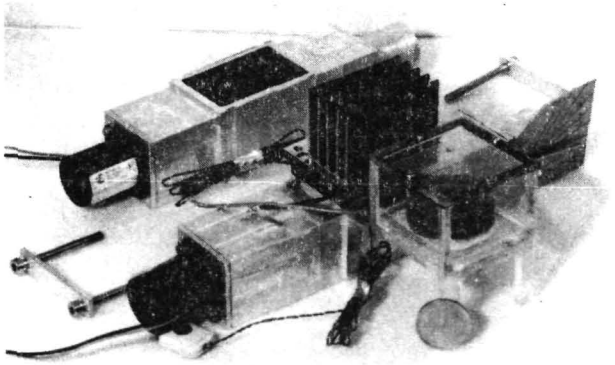

Fig. 15: WRM, disassembled

Details of the design of the wick assembly, the cooling fins and the soil housing can be detected from Fig. 16.

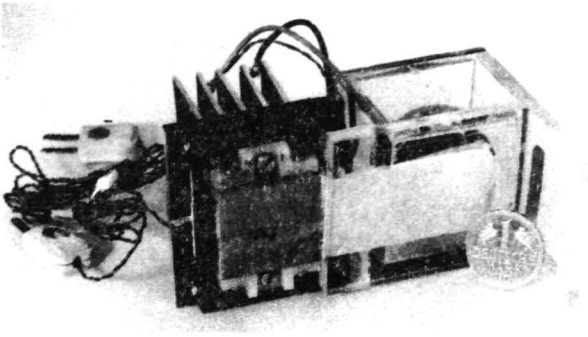

Fig. 16: Detail of wick assembly and its routing into the soil housing. Also shown are the cooling fins

6. TEST SET-UP, INSTRUMENTATION AND CONDITIONS

The test set-up is shown in Fig. 17. As can be seen from the picture, the WRM was placed on an electronic scale in order to determine the weight difference in a certain time period due to the condensate accumulated in the soil.

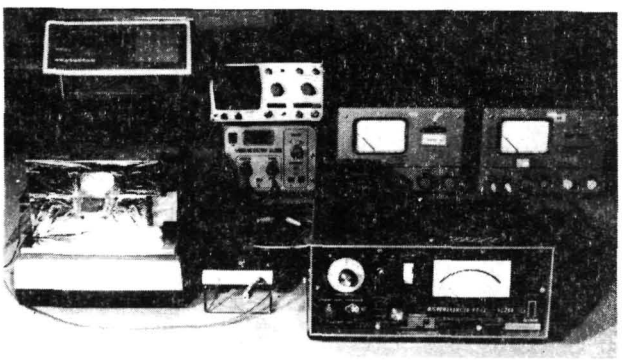

Fig. 17: Test arrangement used for the WRM

Three power supplies were used to provide regulated DC power to the Peltier element, the cold side fan and the hot side fan. Rather than using flow meters, the cold and hot side mass flow rate was determined by means of a delta pressure reading with a micromanometer. The relation between delta pressure and volume flow of the two ducts was determined prior by means of a volume flow meter and the same micromanometer.
Thermocouples have been placed at the following locations:

1. Air, ambient
2. Air, outlet, fin
3. Air, outlet, condenser
4. Condenser grid
5. Peltier element, cold side
6. Peltier element, hot side

Tests were initially performed in a clean room in order to have fairly constant ambient conditions. But due to the comparatively dry air no condensation could be observed. Therefore another room was selected the air humidity of which was artificially increased to approx. 50 % with a rather primitive humidifier. However, this value is still far below the operation range of 80 - 90 % foreseen for the BF.

7. TEST RESULTS AND DISCUSSION

Since the detailed test data reduction is described in (2) and (3), only the test results shall be discussed below.
Fig. 18 shows the condensation rate as function of the cold side mass flow rate.

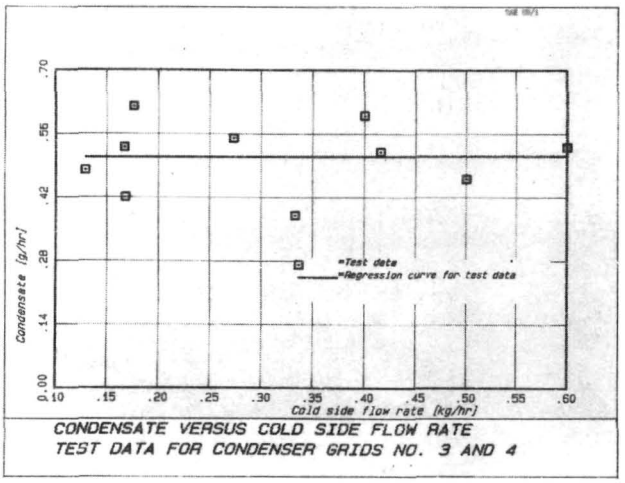

Fig. 18: Condensate versus cold side flow rate. Test data for condenser grid no. 3 and 4.

Despite of the considerable scatter it is apparent that the device functions as expected.
The power of the Peltier element was $N_{el,P}$ = 6 W, that for the fan $N_{el,F}$ = 0.36 W, the measured condensation rate $\dot{G}_W$ = 0.51 ± 0.08 g/hr, hence the measured performance $N_{el,P+F}/\dot{G}_W$ = 12.5 W/(g/hr).

If one compares that value with the theoretical performance of 6.3 W/(g/hr) as predicted in section 3 one must conclude that the design has to be refined in order to further reduce heat leaks.
Fig. 19 illustrates the measured condenser temperatures and again no significant variation with the cold side mass flow can be observed.

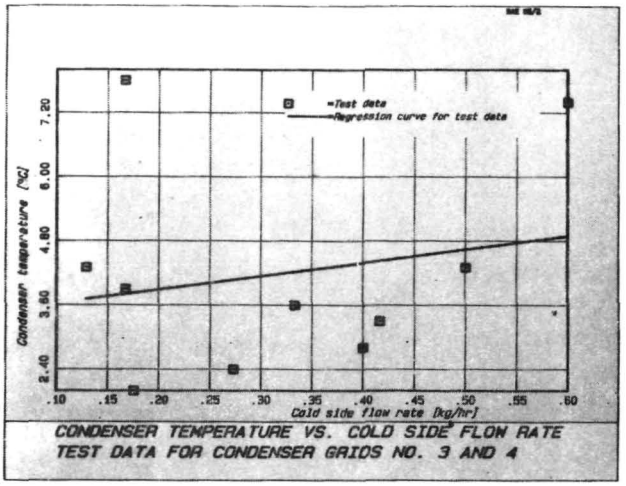

Fig. 19: Condenser temperature versus cold side flow rate. Test data for condenser grid no. 3 and 4.

Fig. 20 depicts the variation of the temperature difference between the condenser grid and the the cold side of the Peltier element with the cold side mass flow rate.

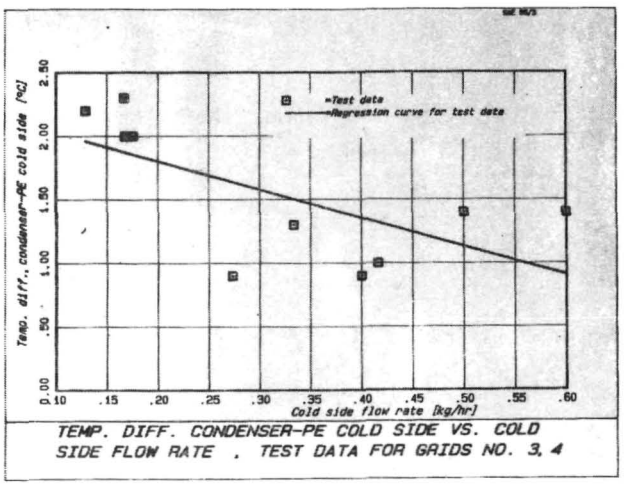

Fig. 20: Temperature difference between Peltier element cold side and condenser grid versus cold side mass flow rate. Test data for condenser grid no. 3 and 4.

As can be seen from the figure, that difference is in the order of 1.5 °C (2.7 °F) and allows therefore the conclusion that the thermal contact between the cold side of the Peltier element and the condenser grid is quite good.

Fig. 21 shows the difference between the air at the condenser outlet and the condenser grid.

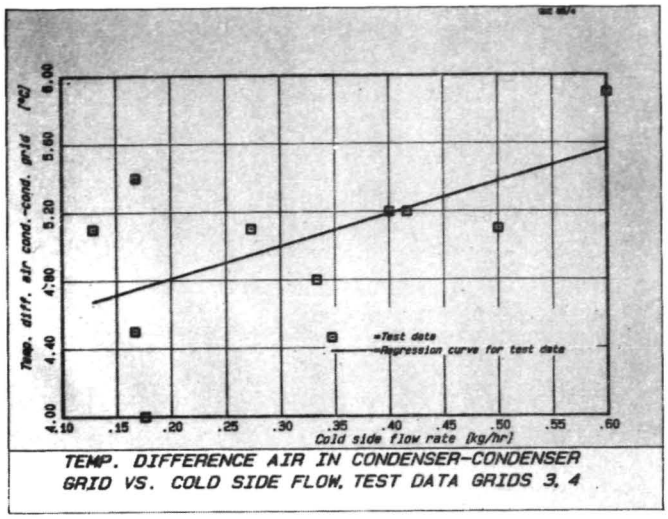

Fig. 21: Temperature difference between the air at the condenser outlet and the condenser grid versus cold side mass flow rate. Test data for condenser grid no. 3 and 4.

Appr. 5 °C (9 °F) are observed, a fact which clearly indicates that in a future design the transfer area should be significantly increased in order to reduce the temperature difference.

Finally, Fig. 22 illustrates that the temperature difference between the Peltier element hot side and the outlet air is appr. 7.5 °C (13.5 °F). Clearly, design improvements are necessary also in this area.

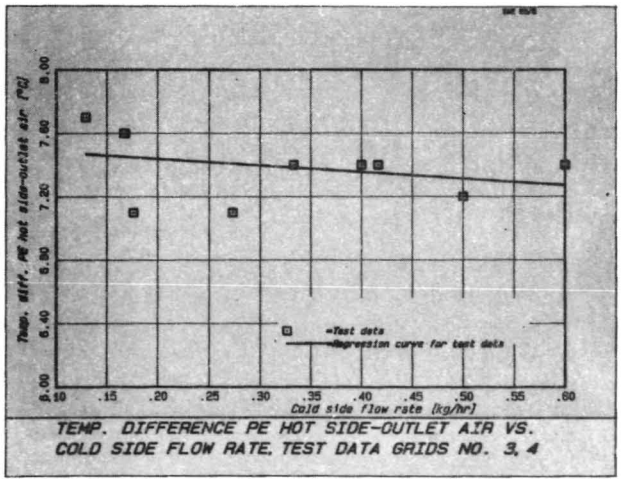

Fig. 22: Temperature difference between hot side of Peltier element and outlet air versus cold side mass flow rate. Test data valid for condenser grid no. 3 and 4.

8. SUMMARY AND CONCLUSIONS

- o A concept for the Life Support Subsystem of the BOTANY FACILITY is described which for the task of the humidity control of the atmosphere is based on water reclamation. Such reclamation is obtained by using a Peltier element and a condenser/wick assembly by which elements the water removed from the air is routed back into the soil supporting the plant samples.

- o This reclamation concept is applicable to cuvettes (= plant growth chambers) flown in micro-gravity and 1 g environment but it are the former which appear technologically more sophisticated.

- o By simple analyses an estimate of the specific power consumption (Watt/g/hr condensate) is provided in order to see if the resulting power consumption is compatible with the power budget allocated to the BOTANY FACILITY.

- o In order to prove the feasibility of the concept, a pre-prototype of a water reclamation module has been designed, manufactured and tested.

- o The test results confirm the viability of the concept. They show on the other hand that a very careful thermal design of the Peltier element/waste heat rejection/condenser/wick assembly is required to minimize heat leaks and to come at least close to the theoretical value of the specific power consumption in terms of Watt per g/hr condensate.

9. NOMENCLATURE

ASACC	:	Atmosphere Storage And Composition Control
BF	:	Botany Facility
COP	:	Coefficient of performance
$\dot{G}$	:	Mass flow rate in kg/hr (air) or g/hr (water)
LSS	:	Life Support Subsystem
N	:	Electrical power consumption (W)
$\dot{Q}$	:	Heat load (W)
VASAH	:	Ventilation And Soil/Air Humidification
WRM	:	Water Reclamation Module
c_p	:	Specific heat at constant pressure
r	:	heat of evaporation
t	:	Temperature in degree C (degree F)
x	:	Air water content in g water per kg dry air

10. SUBSCRIPTS

A	:	Air
C	:	Condenser
F	:	Fan
H	:	Heater
P	:	Peltier element
W	:	Water
i	:	inlet
l	:	latent
o	:	outlet
s	:	sensible

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TN-RT 554-051/84

12. ACKNOWLEDGEMENT

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Wet-Oxidation Waste Management System for CELSS

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ABSTRACT

A wet oxidation system will be useful in CELSS as a facility to treat organic wastes and to redistribute inorganic compounds and elements. However at rather higher temperatures needed in this reaction, for instance, at 260 °C, only 80% of organic carbon in a raw material can be oxidized, and 20% of it will remain in the liquid mainly as acetic acid, which is virtually non-combustible. Furthermore, nitrogen is transformed to ammonium ions which normally cannot be absorbed by plants. To resolve these problems, it becomes necessary to use catalysts. Noble metals such as Ru, Rh and so on have proved to be partially effective as these catalysts. That is, oxidation does not occur completely, and the unexpected denitrification, instead of the expected nitrification, occurs. So,it is essential to develop the catalysts which are able to realize the complete oxidation and the nitrification.

MANY RESEARCH EFFORTS have been funded to make wet oxidation systems commercially available for the purpose of treating organic wastes, particularly sewage sludge, and since 1957, the Zimmermann Process (Zimpro) has been successfully used in the USA, Japan and other countries. This system seems useful in a Closed Ecological Life Support System (CELSS) to treat and reduce organic components of wastewater from plants, human beings and so on, into inorganic compounds (and elements).

But it seems that the discussions on the applicability of wet oxidation to CELSS does not fully reflect the experiences and results obtained in this field. This paper will discuss the usefulness and the unsolved problems of wet oxidation in CELSS on the basis of authors' ten-year research on wet oxidation of sewage sludge.

EXPERIMENTAL METHODS

Wet oxidation is a reaction in which organic or reductive compounds are oxidized in the presence of liquid water. This reaction occurs between 100°C and 374°C which is the critical temperature of water, so the reaction should be carried out in a pressure vessel (autoclave) to prevent evaporation of water.

All data shown later are the results of autoclave tests. The pressure in the autoclave at a given reaction temperature is determined as a sum of the pressures of water and gases which are introduced into the reactor at a room temperature before the beginning of the experiment. Therefore, it is impossible to keep the reaction pressure below a saturated vapor pressure of water at the given reaction temperature.

Before an experiment,it is necessary to introduce enough oxygen gas into the reactor so as to accomplish the intended oxidation. The rate of oxygen gas in the reactor to the chemical oxygen demand

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(COD) of a raw material is called "added oxygen index(R)". In the case of R=1, it means that the exact quantity of oxygen has been added to oxidize the raw materials completely.

Pure oxygen gas or a mixture of oxygen and nitrogen gases has been used. Even after the determination of "R", it is possible to exchange the gas ratio of oxygen to nitrogen at will. You have only to increase or decrease the volume of the raw material. Sewage sludge is used as a raw material.

EFFECTS OF THE REACTION TEMPERATURE

CARBON - Fig.1 shows the relation between the reaction temperature and total organic carbon(TOC).The temperature is increased from 110°C to 310°C at an interval of 50°C. Pressures are all set at 75kgf/cm^2 except in the case of 310 °C, where the pressure is controlled at 115kgf/cm^2.The reason why high pressures are applied at lower temperatures of 110 °C or 160°C will be discussed later. The reaction time, after the temperature of the reactor reached the designed temperature, is 30 minutes. Added oxygen index(R) is unity and pure oxygen is used as an oxygen source.

Total organic carbon[TOC(mix)], filtrable organic carbon[TOC(filt)] and converted carbon quantities of acetic acid, C_2-C_6 organic acids(the sum of acetic, propionic, i-butyric, n-butyric, i-valeric, n-valeric, i-capric, n-capric acids) in oxidized liquors are shown in fig.1 as the percentage of carbon quantities of the raw material versus the respective temperature.

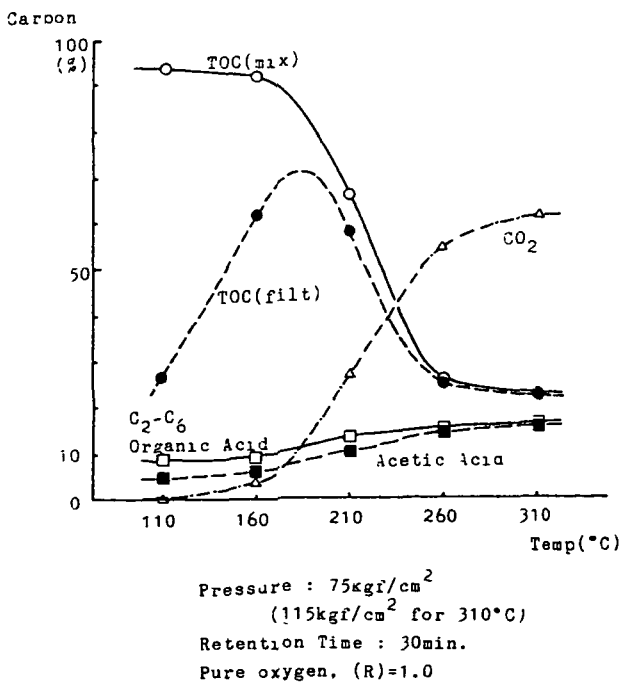


Fig.1 Reaction Temperature and TOC

The behaviour of TOC(mix) curves in the figure shows that the oxidation occurs when the temperature is higher than 160°C, and this process becomes more active at the higher temperature. However when the temperature exceeds 260 °C, the reaction will not proceed any more. The value of TOC(filt) increases with the increase of temperature and passes through the highest value in the range of 160 °C to 210 °C, then it decreases and approaches to the curve of TOC(mix) after 210°C.

In the case where the raw material is sewage sludge, solubilization of suspended organic compounds occurs first at a temperature less than 160 °C, then both solubilization and oxidation proceed in between 160°C and 210°C, and after 210°C oxidation only occurs.

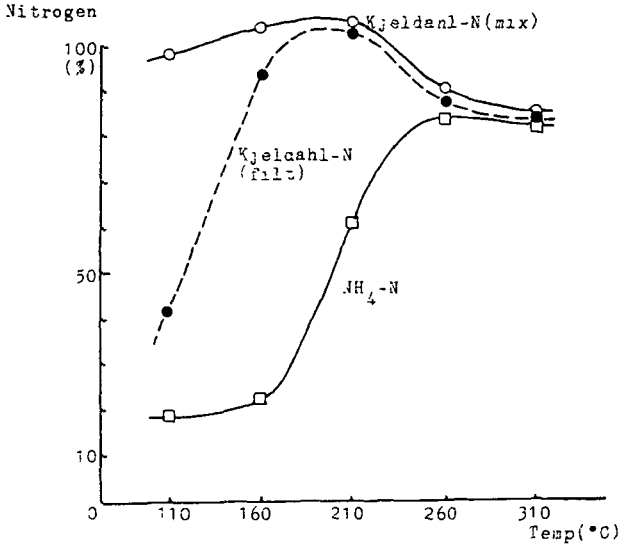
With the increase of reaction temperature, amount of C_2-C_6 organic acids increases and their quantity reaches two-thirds of TOC(mix) at temperatures higher than 260°C. On the other hand, the ratio of acetic acid in C_2-C_6 organic acids increases with the increase of the reaction temperature. When it reaches 260 °C, acetic acid occupies nearly the total amount of C_2-C_6 organic acids, and maintains this amount independently to the increase of temperature thereafter.

This does not mean that the reaction had reached equilibrium. Acetic acid, whose concentration ranges from zero to 16g/l as converted carbon quantities, is not oxidized at all, even if excessive oxygen is added to the reactor. Therefore the main products in wet oxidation is "acetic acid" in addition to carbon dioxide and water.

NITROGEN - Fig.2 shows the relation between temperature and nitrogen output. As the temperature becomes higher, the solubilization of suspended nitrogen compounds occurs, and it is almost

completed at 160 °C. But this solubilization does not result in the formation of ammonia. Between 160°C and 210°C, at which both the solubilization and the oxidation of organic compounds occur, production of ammonia increases rapidly. Even if the solubilization has been completed, soluble nitrogen compounds do not become ammonia totally at 210 °C, but at 260°C.

In wet oxidation without catalysts, the nitrogen compounds may not be oxidized to nitrite and nitrate. On the other hand, forms of nitrogen usable by plants are nitrite and/or nitrate with the exception of some plants such as rice. So in CELSS, it is impossible to apply oxidized liquor directly to plant culture in hydroponic solutions. It is

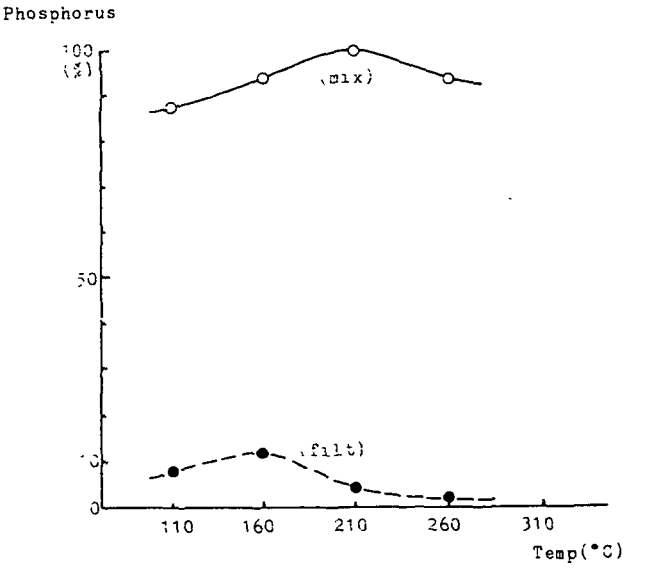


Pressure : 75kgf/cm^2
(115kgf/cm^2 for 310°C)
Retention Time : 30min.
Pure oxygen, (R)=1.0

Fig.2 Reaction Temperature and Nitrogen

necessary to convert ammonia to the forms nitrite and/or nitrate by catalysts or by any other means. The decrease of Kjeldahl -nitrogen above 260°C is due to either the denitrification or NOx formation.

PHOSPHORUS - Fig.3 shows the relation between the temperature and phosphorus output. The percentage of total and soluble(filterable) phosphorus in the oxidized liquor to the quantity of raw material versus the respective



Pressure .75kgf/cm^2
(115kgf/cm^2 for 310°C)
Retention Time : 30min.
Pure Oxygen, (R)=1.0

Fig.3 Reaction Temperature and Phosphorus

temperature is shown. The ratio of phosphorus in the liquid to the total phosphorus quantity is remarkably small comparing with the one of nitrogen in the liquid to the total nitrogen quantity, which gives only approximately 10 per cent at best. This may be caused by the fact that sewage sludge contains various cations such as calcium ions which produce unsoluble salts with phosphorus. In CELSS, the wet oxidation should function to re-supply nutrient sources for plant culture in hydroponic solutions. It is rather desirable for nutritious elements such as phosphorus, nitrogen etc. to be dissolved in water. Therefore, this phenomenon is not favourable in making use of wet oxidation in CELSS. The behavior of phosphorus in wet oxidation has not been elucidated except in the case of sewage sludge. In this respect, the study of phosphorus is greatly needed.

EFFECTS OF THE REACTION TIME

CARBON - Fig.4 shows the status of TOC along with time. The reaction temperatures are 210°C and 260 °C. In either cases, the percentage of TOC(mix)

and TOC(filt) to the quantity of a raw material is shown in relation with time. Pressure is kept 35kgf/cm^2 in the case of 210°C, and 75kgf/cm^2 in the case of 260°C. The added oxygen index(R) is unity, and pure oxygen is used as a oxygen source. As this is a batch process, time is needed to reach the designated temperature. In order to know the changes of TOC in the heating process, the quantity of TOC is checked at the temperature of 160°C. The results are shown in the same figure.

The oxidation at 260 °C occurs markedly in the initial 15 minutes. But after that, the reaction curve declines gradually. Fig.4 indicates that, only 80 per cent of TOC(mix) is oxidized at 260 °C, even if the reaction time exceeds 45 minutes. The solubilization has finished before the temperature of the reactor reaches the designated temperature. As a result, TOC(filt) quantity will be the same as TOC(mix).

At 210°C, only 15 per cent of TOC (mix) is oxidized at zero minute on the horizontal axis of reaction time in fig.4. The decrease of TOC(mix) is remarkable during the first 15 minutes, even if it does not show so rapid a decrease as seen at 260°C. After that, the oxidation curve declines gradually. The solubilization of TOC reaches a plateau status after the 15-minute process. But suspended TOC, i.e. TOC(mix) minus TOC(filt), does not disappear even after 45 minutes.

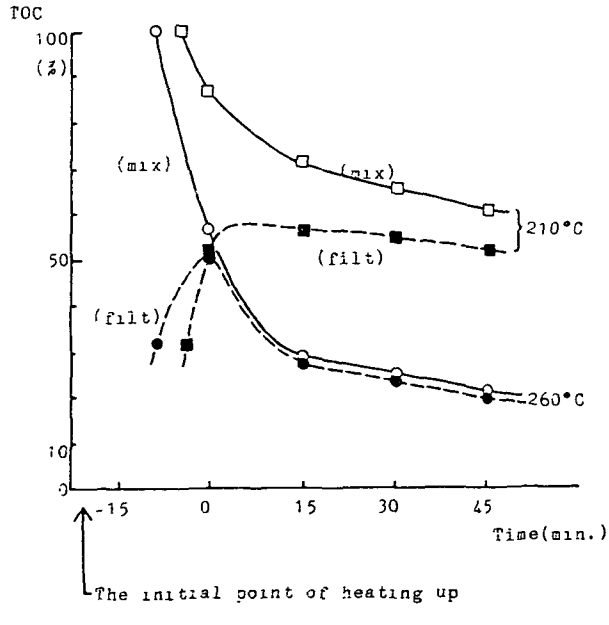


Fig.4 Reaction Time and TOC (1)

TOC(mix),TOC(filt) and the converted carbon quantities of acetic acid and C_2-C_6 organic acids are shown in fig.5 in relation to their percentage to raw materials versus a time. The reaction temperature in this case is 260 °C. The other experimental conditions are the same as in fig.4. As it was explained previously, the solubilization of TOC has finished before the reaction temperature reaches the designated temperature. C_2-C_6 organic acids reach their maximum within

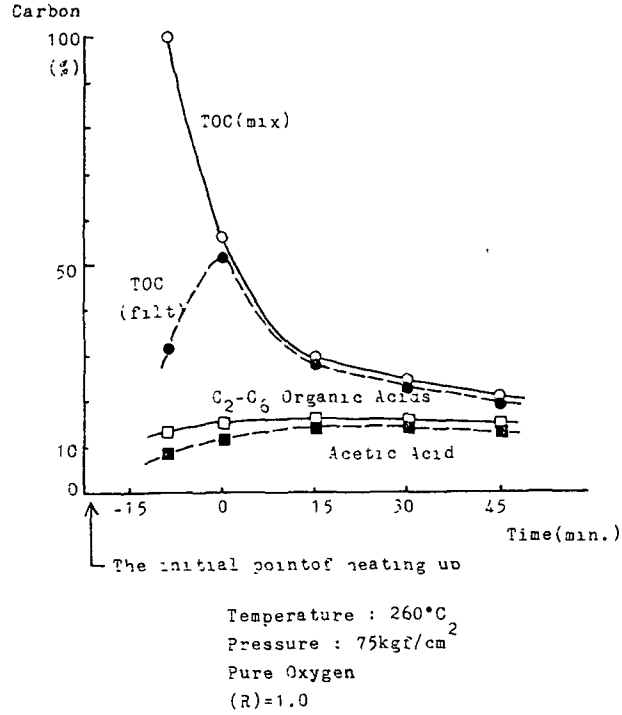


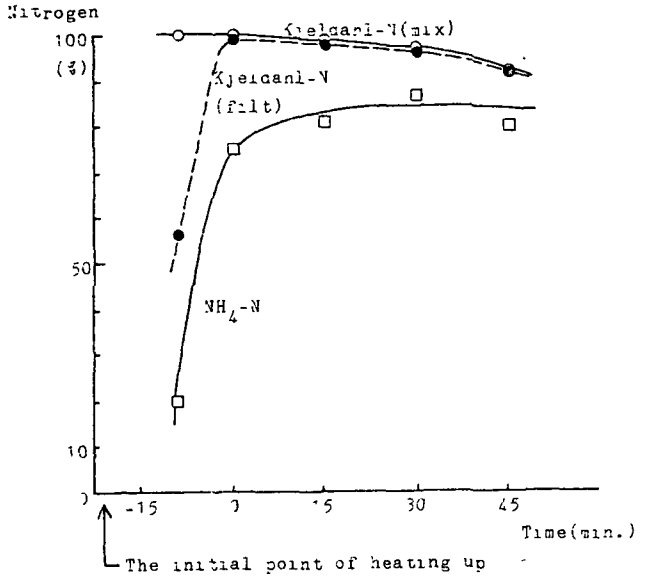
Fig.5 Reaction Time and TOC (2)

15 minutes and do not decrease any more despite that TOC(mix) decreases. The ratio of acetic acid to C_2-C_6 organic acids increases with time until 30 minutes but does not decrease even after 45 minutes.

Fig.4 and 5 show that the reaction temperature determines the upper limit of the oxidation. Considering the results of fig.1 together with fig.4 and 5, it is understood that only 80 per cent of organic compounds in raw materials is oxidized even at the reaction temperature of 310°C, and the remaining 20 per cent is non-combustible and consists mainly of

acetic acid.

Probably, acetic acid will not be oxidized at more than 310°C. In CELSS, it is desirable that organic compounds in a raw material are wet-oxidized completely, and then it is necessary to develop the catalysts which have capability to oxidize acetic acid completely or to change the course of the reaction of wet-oxidation without producing acetic acid.



Temperature : 260°C
Pressure : 75kgf/cm^2
Pure Oxygen
(R)=1.0

Fig.6 Reaction Time and Nitrogen

Nitrogen - Fig.6 shows the status of the nitrogen over time. The conditions in fig.6 are the same as the ones of in fig.5 experimentally and materially. The solubilization of the suspended nitrogen compounds has finished before the reactor has reached the designated temperature. The dissolved nitrogen compounds has led to ammonia in the same time. After the designated temperature has been realized, the Kjeldahl-nitrogen curve declines gradually with time. The percentage of ammonia to the Kjeldahl-nitrogen forms the plateau status after 15 minutes.

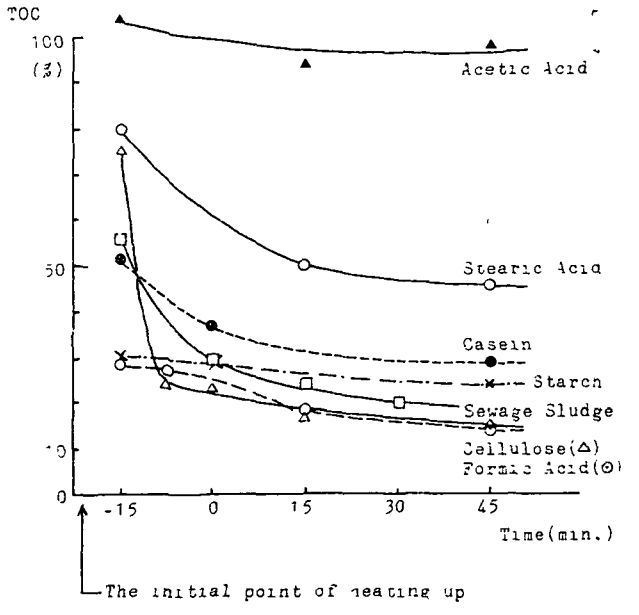
TIME-COURSE OF VARIOUS KINDS OF MATERIALS

Fig.7 shows the time-course of various kinds of materials in wet oxidation. Each material is wet-oxidized under the conditions of 260 °C and 75kgf/cm^2. Pure oxygen is used as an oxygen source. As discussed previously, acetic acid is virtually non-combustible, so only a few percent of acetic acid are oxidized. About 50 per cent of stearic acid is oxidized in an hour. Casein, sewage sludge and cellulose show similar characteristics to one another in the time-course curves. It can be easily recognized by their positions in the figure that formic acid and starch are easily oxidized.

It is also known that cyanates in raw materials are oxidized to ammonia at 100 per cent, and 0.01 per cent of it remains in gas. Sulfur, sulfide or sulfite, is oxidized to sulfate and remains in liquid. Sodium, potassium and chlorine ions are intact under the wet oxidation, and remain in liquid.

There are various kinds of materials, some are easy to oxidized, and some are not. The research of wet oxidation in the CELSS project should start based on the elabolate choice of suitable raw materials.

In CELSS, raw materials will be



Temperature : 260°C
Pressure : 75kgf/cm^2
Pure oxygen

Fig.7 Time-course of Various Kinds of Materials

kitchen and laundry wastewater, feces, non-edible parts of plants, used hydroponic solution, trash and so on. They are entirely different from sewage sludge in character. Although the results of wet oxidation studies of sewage sludge cannot be applied directly to CELSS, because the fundamental phenomena are the same, the results of wet oxidation studies of sewage sludge can be of reference to the studies of CELSS.

BEHAVIORS OF METALS

The author has not yet studied the behaviour of metals in wet oxidation experimentally, but has done so in the field. The studies of a wet oxidation facility of sewage sludge in the Yokohama north-side-sewage-treatment plant, whose operating conditions are 240 °C in temperature, 72 kgf/cm^2 in pressure and 1 hour in retention time, show that most of Cd, Zn, Cu, Pb, Cr, Mn or Fe contents in a raw material are transferred to solid after the wet oxidation.

One man, even in a normal life, usually uptakes daily 0.05, 10.9, 5.85, 2.77, 0.177, 0.354, 0.022mg of Cd, Zn, Mn, Cu, Pb, As, Hg respectively. These are excreted and urinated daily at 1000 to 1200mls. It is unknown how much part of the respective element will be in liquid or solid after the wet oxidation. Therefore, trial oxidation in the CELSS project should be carried out after the studies of the metal behaviors have been established.

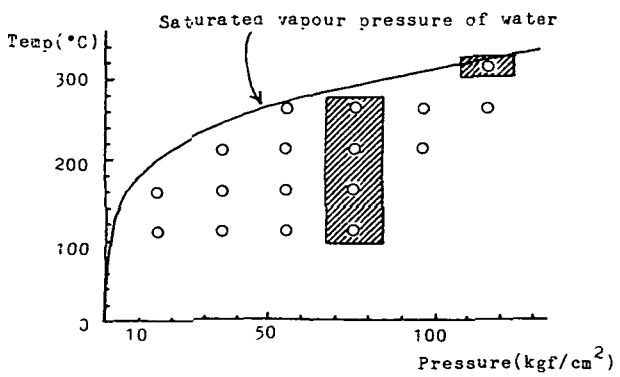


Fig.8 Saturated Vapour Pressure of Water

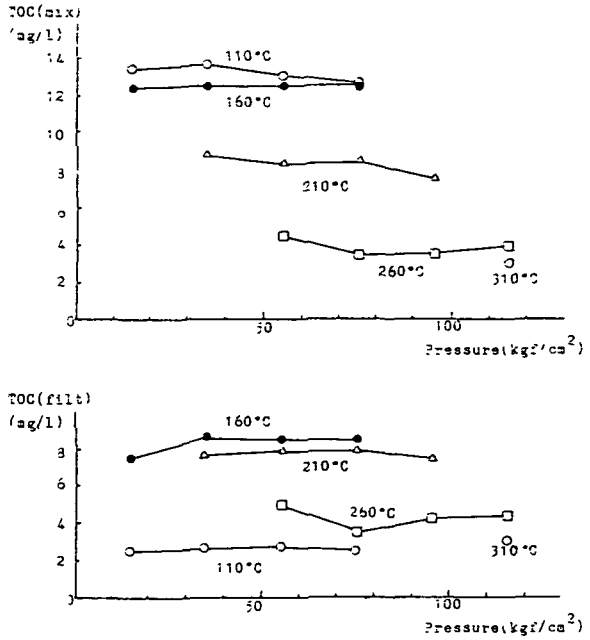


Fig.9 Pressure and Oxidized Ratio

PRESSURE AND OXIDIZED RATIO

Reaction pressure of the wet oxidation is determined as the sum of saturated vapor pressure of water at a certain reaction temperature and the pressure of gases which were introduced into the reactor at room temperature before the beginning of the experiment.

Fig.8 shows a curve of a saturated vapor pressure of water. A reaction cannot take place in the conditions of any temperature-pressure combination above the curve. Experiments to study pressure effects are carried out under the conditions of temperature-pressure combinations below the curve shown in the figure(*). The reaction time is 30 minutes and the added oxygen index(R) is unity. The results of tests executed under these combinations of condition are shown in fig.9.

Fig.9 shows that the oxidized ratio depends on the reaction temperature only. This fact is true also in the case in which a mixed gas of oxygen and nitrogen is used as an oxygen source. It means that a pure oxygen gas is not a necessary material as an oxygen source in the wet

(*)The experimental conditions adopted in fig.1-3 are the ones enclosed by the hatched area in this figure.

oxidation. In other words, the essential fact is to supply a necessary quantity of oxygen in either pure or mixed status. This fact may be favorable to CELSS.

EFFECTS OF CATALYSTS

As discussed earlier, organic compounds in raw materials can not be oxidized completely and nitrogen compounds remain in liquid in the form of ammonia in the wet oxidation. If and when a wet oxidation facility is used in CELSS, it is desirable that carbon in organic materials shall be involved in a gas recycling system in a form of carbon dioxides and that nitrogen in them is transformed to plant-available nitrates, by completing the oxidation process. In order to realize the above process in a short time without by-products, it is supposed that there would be no alternative way besides utilizing certain catalysts for the time being.

From a sewage-sludge-treatment point of view, the authors have been engaged in research to develop the catalysts so that the wet-oxidation system can exhibit a similar function to a usual combustion furnace system, and remove nitrogen from liquid.

As the result of surveys, it has become clear that noble metals such as Pt, Pd, Rh and Ru among the transition elements are hopeful as catalysts. These metals are needed to be supported on carriers made of alumina and titania etc.

To prevent suspended particles in liquid from poisoning a catalyst, filtrated-wet-oxidized-sewage-sludge output is used in the wet oxidation tests on the catalysts. One catalyst has been used 20 times in wet oxidation of fresh filtrates. The results are as follows.

When a Pt or Pd catalyst is used, nitrogen in the liquid is denitrified at 100 per cent. When a Ru or Rh catalyst is used, a half of nitrogen in the liquid is denitrified. The rest remains in the liquid as a nitrate when Ru is used and as ammonia when Rh is.

On the other hand, organic compounds are not catalytically oxidized at all, when Pt is used. The wet-oxidized output of TOC decreases to a half of a control, when Rh is used. The oxidation catalysis

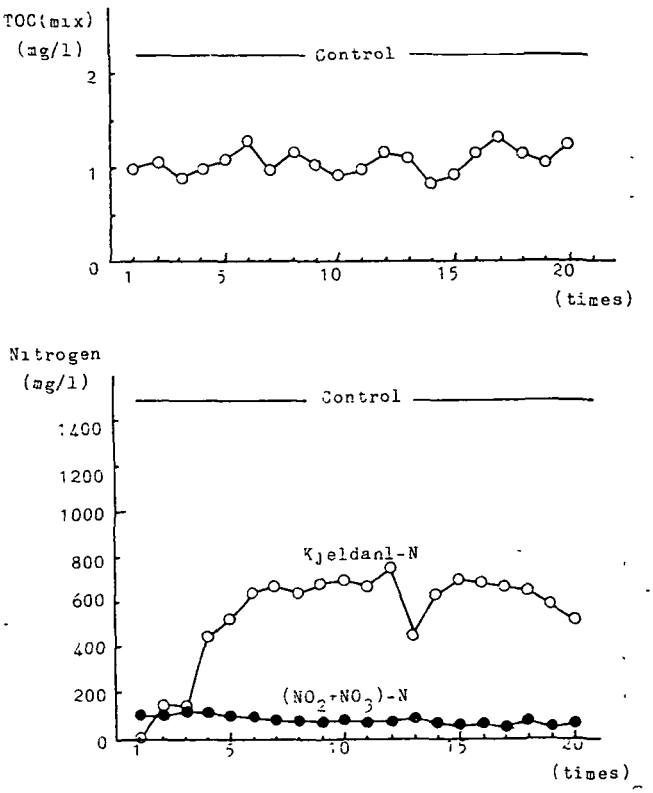


Fig.10 Effects of Rh Catalyst

of Ru or Pd is better than that of Pt and worse than that of Rh. The result of Rh is shown in fig.10.

Good catalysts for both oxidation and nitrification have not yet been found up to now. Therefore, research should be initiated to develop the catalysts which shall be involved in a recycling system of CELSS, will not be poisonous to working staff as well as living things in a space station, and shall have a effective catalysis for oxidation and nitrification.

CONCLUSIONS

The behaviors of various kinds of materials in wet oxidation were shown and the applicability of wet oxidation to CELSS was discussed. In Japan, a wet-oxidation facility for CELSS has been designed already. In this regard, the following projects are to start promptly:

(1)To manufacture a trial wet-oxidation facility,

(2)To execute tests of wet-oxidation for selected materials which are expected to be used in practice, and

(3)To observe the behavior of the

materials.

The complete solubilization of raw materials will be a desirable way from the element-redistribution point of view. On the other hand, in the case of unsoluble phosphorous compounds, there would be no problem if they should be sprinkled to a plant culture in a hydroponic solution, and , at the same time, if the plant culture would be able to utilize them as nourishment.

In this respect, studies and discussions to confirm if each element, in a wet-oxidation output, is desirable in a liquid, solid or a gas phase. Thus, further studies on chemical-form controlable catalysts of each element can commence effectively.

Acknowledgement

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SIMULATION MODEL FOR PLANT GROWTH IN CONTROLLED ENVIRONMENT SYSTEMS

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INTRODUCTION

Among the requirements for a self-sustaining regenerative CELSS are atmospheric regeneration by reduction of carbon dioxide and evolution of oxygen and reincorporation of the reduced carbon into the organic constituents of human food. A controlled-environment crop production system is a probable component in accomplishing these requirements. However, higher plants, together with their reactions to environmental and cultural conditions, comprise an extremely complex biological system, and inclusion of a crop production system in a CELSS requires answers to questions of its reliability and predictability to meet the regenerative requirements in response to perturbations in the environmental conditions and its flexibility for controlled levels of functioning in optimization of mass flows within a CELSS in response to selected environmental conditions. Two concerns in evaluation of crop reactions to environmental conditions are how to evaluate effects of environmental conditions on crop growth and yield, and once any set of environmental conditions has been experienced by the crop, what can be done to readjust crop growth and yield to levels for optimization of the CELSS. The capability to respond to the latter concern depends on the ability to evaluate probable outcomes of possible management strategies, including selections from among available environmental conditions. Mathematical modeling of the crop system can assist in addressing these questions and in interpreting the results of discrete experimental studies of crop reactions to environmental conditions.

The crop system that must be addressed is one of a community of plants growing under conditions selected by management and by possible malfunctions. But the complexity of the system makes it inherently difficult to define. The complexity of the crop production system is manifested in the large number of interacting variables needed to describe the inner workings of the system, in the large number of possible behavioral responses and in the large number of environmental regimes to which the system might be subject. Moreover, while the system itself can be highly complex, discrete experiments designed to provide information about the behavior of the system can be only as complex as will allow interpretation. In this context, then, the problem of evaluating the behaviors of crop production systems as a component of a CELSS is how to use a finite and relatively small number of experimental observations, made under relatively simple defined conditions, to make inferences to a nearly infinite set of possible conditions for a vastly more complex system.

THE ABSTRACTION HIERARCHY

In addressing the problems of a crop production system within a CELSS, it is useful to draw on the concept of abstraction hierarchy (Mesarovic et al., 1970) in which the levels of hierarchy refer to how coarsely or finely ground the system is taken to be. The following levels might be identified: (a) CELSS crop ecosystem level; (b) plant community level; (c) single plant level; (d) organ structures and elementary functional processes such as photosynthesis, respiration, translocation, etc., (e) elementary structures such as cells, membranes, local structured processes, etc.; (f) molecular physiology and chemistry; and (g) atomic and molecular physics. Each of these levels is meant to stand for a collection of subsystems or components that co-exist and

possibly interact within the level, and the next higher (coarser) level can be viewed as a level of organizational structure of these components (Gold and Raper, 1983). If one proceeds within the hierarchy from bottom to top, or finer to coarser levels, it becomes increasingly more difficult to control the experimental environment of the system. The breakpoint in biological systems comes at about the single plant level, for which it is possible to control the environment within phytotron and growth chamber facilities. (A phytotron is defined as an assemblage of controlled-environment chambers and glasshouses used simultaneously and in various combinations to investigate plant responses to environment, and the term thus implies function rather than size of the controlled-environment facility.) Thus, while inferences from experiments are desired for CELSS at least at the plant community level, or possibly higher at the crop ecosystem level, the observational level at which experimental conditions can be controlled is limited to that of single plants or very small groups of plants. The processes, such as photosynthesis, respiration, and translocation, that give rise to these observations and upon which extrapolation to higher levels must be based, are at yet a finer level. The ability to make inferences about the class of systems at the complex level of organization is, therefore, dependent upon the ability to draw relationships across levels in the hierarchy. Since the hierarchial structure is an artifact to facilitate scientific description of the system, the usefulness of such a description hinges on the ability to describe each of the levels and each of the subsystems at a given level independently of the other levels and subsystems.

As one proceeds up the hierarchy, it should be noted that the detail necessary to describe a lower level is specifically not wanted for the

description of a higher level (Gold and Raper, 1983). For example, consider the relationship between each component molecule and the aggregate content of carbon dioxide gas within the crop production system. Each of the $6.02 \cdot 10^{23}$ individual molecules within a mole of gaseous carbon dioxide are characterized by their individual positions and energy levels at any instant. While the extent of this clearly is more than one particularly wants to deal with for an aggregate description, the total collection of carbon dioxide molecules can be characterized by definition of volume, temperature and pressure. While these three variables that describe the high level are functions of the infinitely more extensive collection of variables that describe the lower, these functions have no inverse and informational detail is lost as one moves from a finer to a coarser level within the hierarchy. One role of a mathematical model of the crop system thus is to assist in expressing the relevant information functions and determining what detail needs to be retained to characterize the plant growth within the system.

While the behavior of a system at a higher level of abstraction is a function of behavior at the lower level, the lower level operates under constraints imposed by the organizational structure of the higher level (Mesarovic et al., 1970). In a biological system there is the additional complication that part of the behavior of the system at any level is to alter its own structure to cause a feedback relation between constraints and processes. An example of such an interlevel dependency is the rate of photosynthetic assimilation of energy by the plant. The leaf structure of the plant imposes a higher level on the interception and absorption of radiant energy as the input for the photosynthetic process during a time interval. During the time interval the

structure changes in response to the production of photoassimilates available for growth.

Several implications can be drawn from these general arguments about the relationship between levels of the hierarchial structure (Gold and Raper, 1983). First, interactions between subsystems at the same level tend to proceed through the interaction of lower level component processes such as the interaction between a plant and the aerial environment. The processes of carbon dioxide movement within and between the two components is governed by the constraints on molecular movement within each component and the structural relation between the two (which is a constraint imposed by yet a higher level of organization). Second, subsystems at different levels tend to operate on different time scales since changes at one level are functions of processes at the lower level. As an extreme example, consider morphological features, such as the position of leaves which are developed on a relatively long time scale of days and remain constant over even longer periods of weeks once built into the growth of a plant, and physiological characteristics, such as photosynthetic rate per unit leaf area, which are reversible on a relatively short time scale of minutes or seconds. Thirdly, an observation of the state of the system intrinsically involves an interaction between the system itself and the measuring device and involves processes at a lower level leading up to the observation. For example, attaching a plexiglas cuvette to a leaf to measure net photosynthetic rate by infrared gas analysis alters the microenvironment of the leaf, and the net exchange of carbon dioxide between the cuvette environment and the chloroplasts within leaf cells involves changes in thermodynamic constraints imposed by size and energy fluxes of the cuvette. Finally, because of feedback loops in

biological systems involving process functions (such as photosynthesis) and structural functions (such as leaf development) between levels and the absence of total cognizance of processes within any level, interlevel relationships cannot be derived from extrapolation from one level to another. Rather, interlevel relationships must be derived from interpolation between levels based upon an understanding of the physical laws that govern behavior of the system at the different levels and upon an understanding of the interlevel interactions.

SIMULATION MODEL FOR CROP PLANTS

Describing the dynamic relationships between levels of the system hierarchy is one of the functions of a mathematical simulation model of crop plants. Most crop simulation models are based on models for single plants, sometimes as modified by the presence of other plants. These models express growth and metabolic activity as a function of environmental variables, using mathematical forms suggested by knowledge of basic biochemistry and plant physiology. Verifying the applicability of these mathematical descriptions and determining values of the parameters to use generally required replicated experimentation under as carefully controlled and precisely identified conditions as possible. The conditions for these experiments should not be chosen for the purpose of direct extrapolation of results to a crop production system for CELSS, such as identifying a set of environmental conditions for maximum yield of a crop species. Although such studies are useful in defining the upper limits of productivity for the crop production system, sustained levels of maximum growth and yield do not optimize the entire CELSS. Also, the technology of ground-based experimental systems is likely not to be directly applicable to an

actual CELSS. Rather, conditions for experimentation should be chosen for the purpose of verifying the mathematical formulation of the underlying plant physiology, for the purpose of testing the formulation of how the levels relate to each other, and for the purpose of learning the values of the appropriate parameters (Gold and Raper, 1983).

As an example of model development to relate individual processes to environmental conditions and behavior of the whole plant with possible application to CELSS, consider the simple, deterministic model for plant growth that we are working on at North Carolina State University. A mathematical simulation model is necessarily a simplification of the actual system under consideration in which essential characteristics of the actual system should be mimicked by the model. In this case our objective is to take into account the dominant features of plant physiology to describe plant growth subject to a wide range of variations in environmental conditions.

A general description of the model can be given without going into the mathematical detail (Wann et al., 1978, 1979, 1984). The effects of incident photosynthetically active radiation and ambient carbon dioxide concentration are incorporated directly through the process of photosynthesis and the effects of temperature directly through the processes of photosynthesis, respiration, growth, and aging. Leaves, stems, and roots are defined as separate organ classes during vegetative growth (Figure 1). For each of these classes, compartments are identified for soluble carbohydrate pool, young tissues capable of active growth, mature tissues incapable of active growth but otherwise capable of normal physiological functioning, growth

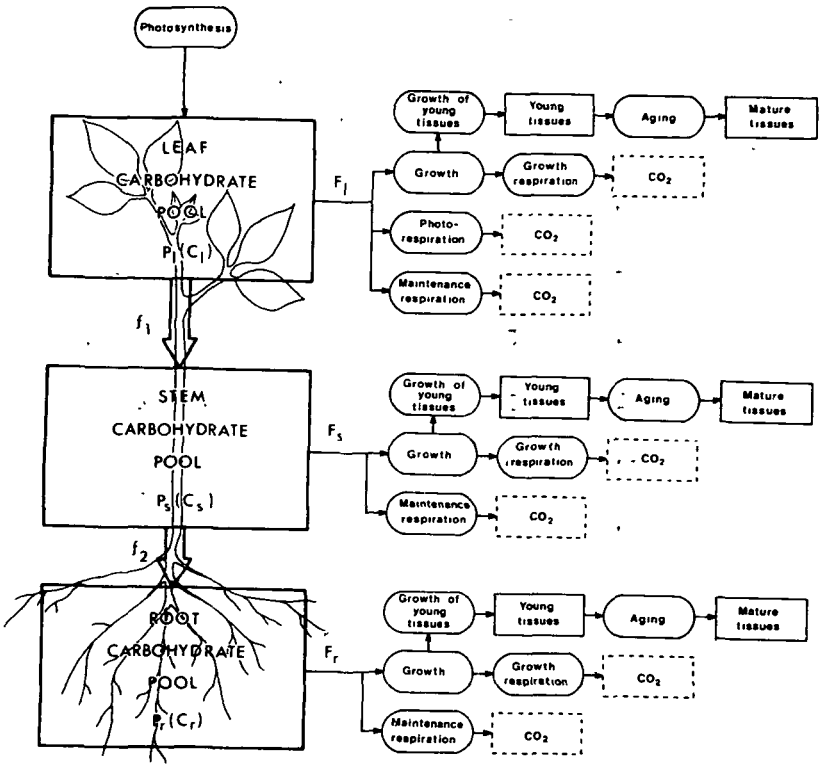


Figure 1. Energy and carbon flows in the plant system as adapted from Wann et al., (1978, 1979, 1984, 1986). Rectangular boxes with solid outline represent compartments within the plant system, rectangular boxes with dashed outline represent compartments external to the plant system, and elliptical boxes represent processes.

and maintenance respiration. An additional compartment for photorespiration is identified for the leaves. Photosynthesis serves as the sole source for the carbohydrate pool in the leaves, and net movement of carbohydrate is from the leaf pool to the stem pool and from the stem pool to the root pool. Since translocation fluxes between pools must be sufficient to maintain a living

state and to support normal growth of the organs, they are determined under two possible conditions (Wann and Raper, 1986). First, when carbohydrate supply in the source (exporting) organ is not limiting, the flux is assumed to be determined by demand of the sink (receiving) organ and by the availability of carbohydrate in the source pool. Source strength is considered as proportionate to the concentration of carbohydrate in the pool of the exporting organ and sink demand is defined as the amount of actively growing tissue in the receiving organ. Conversely, if carbohydrate availability in pools is insufficient to satisfy all demands of growth and respiration by organs, maintenance respiration and growth are assigned the first and second highest priorities for allocation of carbohydrate within the organ pool. The remaining carbohydrate in the source pool is then available for translocation to the sink organ.

Of the five classes of parameters included in the model, only four (the maximum photosynthetic rate, the specific respiration rates, the specific aging rates, and the maximum specific growth rates) are directly dependent on temperature (Wann et al., 1978, 1979, 1984). The fifth class of parameter, the translocation coefficients, are considered to be responsive to temperature indirectly through the effects of temperature on the concentration of carbohydrate in the source pool and the size and metabolic activity of the sink organ (Wann and Raper, 1984).

A set of nonlinear, ordinary differential equations (Wann et al., 1978, 1979) is used to describe the flow of energy (and carbon) through all compartments specified in Figure 1. For a given instant the net flow rates

for each compartment are computed as the difference between input and output flow rates. The entire set of differential equations has simultaneous solution to give a continuous simulation of plant physiological processes. Since the simulation of plant growth is continuous, the distinct conditions of pool sizes, average age of tissues, and metabolic activity that are reflective of the environmental history of the plant do not require updating at discrete intervals of time or growth stage, but are predicted by the performance of the model in response to environmental conditions during growth. Furthermore, this is the only simulation model for growth of the whole plant of which we are aware (Legg, 1981) that the effects of environment are incorporated mechanistically through their efforts on the processes of photosynthesis and respiration.

MODEL VALIDATION

Many of the important parameters required by the model, such as translocation coefficients and aging rates, are difficult to measure directly and can only be estimated by fitting the model against experimental data through the use of numerical integration and iterative weighted least squares techniques. Data initially used in fitting the model were obtained from experiments conducted with tobacco plants (Nicotiana tabacum L. 'NC 2326') grown for 35 days at constant temperature conditons over a range of 14 to 34^{o}C and a constant photosynthetically active reaiation of 750 µmol $m^{-2}s^{-1}$ in growth rooms of the phytotron at North Carolina State University. Plants were sampled at 2 to 3 day intervals during the growth period. The fit of the model was verified by comparing the dry weights of leaves, stems, and roots with the measured dry weights at each sampling date for each temperature (Wann et al., 1978).

The model was then validated against independently derived data sets from experiments with natural variation in radiation under fixed temperatures in controlled-temperature glasshouses of the phytotron (Wann et al., 1978) and from experiments with weekly (Figure 2) and daily changes in temperature under constant radiation in growth chambers (Wann and Raper, 1984). The plants in all programs of weekly temperature change (Figure 2, inserts) were subjected to each of the five day/night temperatures during 1 week of the 5-week growth period, but since the sequence of temperatures was different in each of the ten programs, the plants in each of the programs experienced any single day/night temperature at a different combination of growth stage and previous temperature history. When reacting to a given temperature, the plants thus would be expected to have distinct conditions of pool sizes, average age of tissues, and metabolic activity. These factors are not updated at discrete intervals of time or growth stage for the continuous simulation of plant growth by this model, but reflect the performance of the model. Thus, the generally good agreement between predicted and measured performance of plants to changes in temperature (Figure 2) and radiation (Wann et al., 1978) indicates that the model is capable of responding to variable conditions during plant development.

Although the model was originally developed and validated with experimental data and parameter values for tobacco, it has been adapted for simulation of vegetative growth of soybean (Glycine max (L.) Merr. 'Ransom') with limited modification of parameter values and validated against data sets for plants grown in phototron chambers over a 28-day period at temperatures over the range of 14 to 34^{o}C (Figure 3) and for plants grown in hydroponic culture with independently varied root and shoot temperatures (Figure 4). The good

Figure 2. Simulated (continuous line) and measured (o) dry weights of tobacco plants grown under the weekly changes in temperature schedules shown in insets. Within the insets, the 9-h day temperature is indicated by the continuous line and the 15-h night temperature is indicated by the dashed line. (Adapted from Wann and Raper, 1984.)

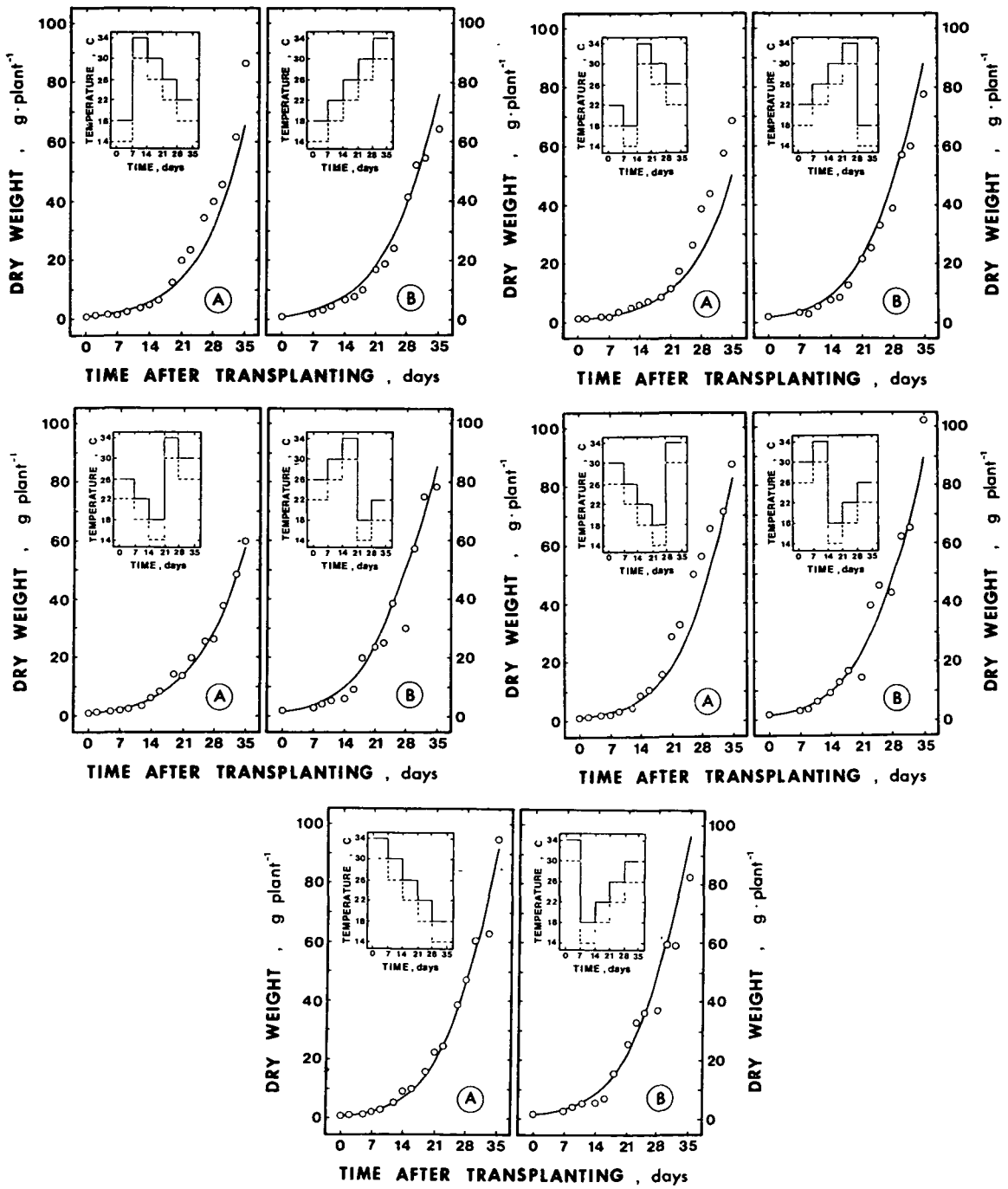


Figure 3. Comparison of calculated and measured dry weights of soybean plants and plant parts under five constant day/night temperatures. Photosynthetic photon flux density during the 9-h day period was 750 μmol s^{-1} m^{-2}. (Adapted from Wann and Raper, 1979.)

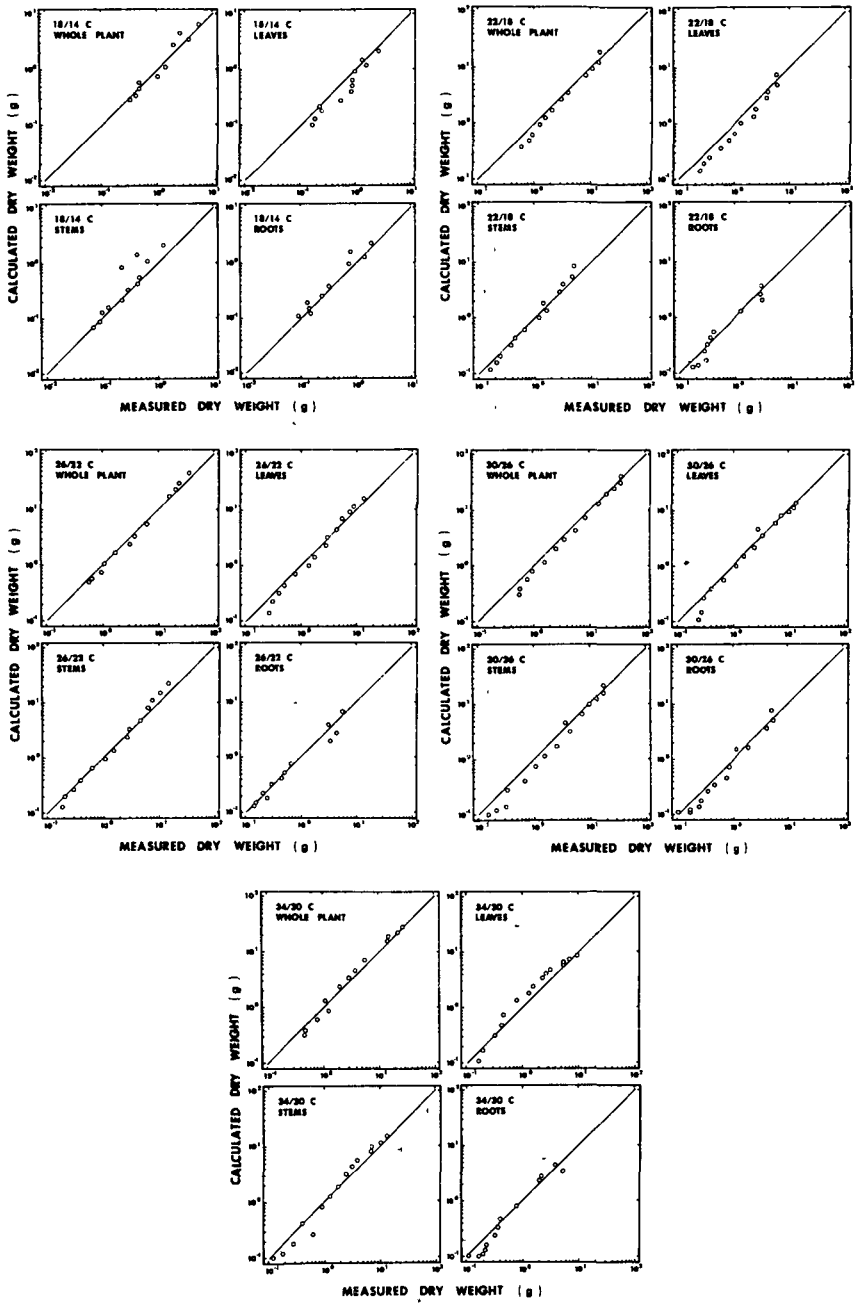
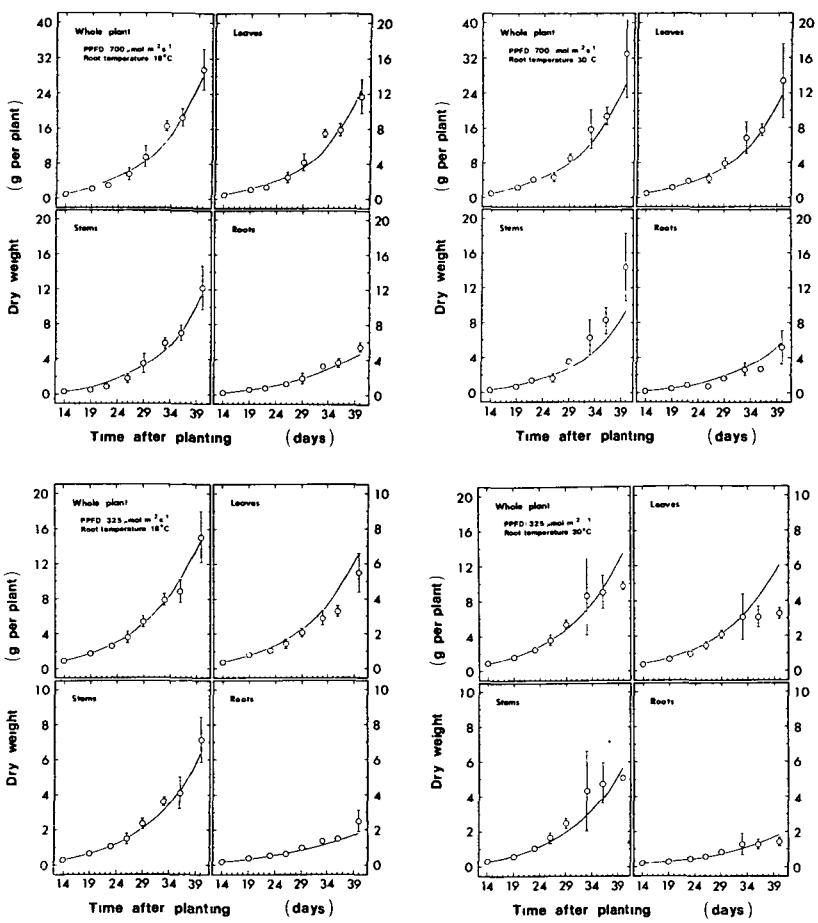


Figure 4. Simulated (continuous line) and measured (o) dry weights of soybean plants and plant parts grown in hydroponic culture at root-zone temperatures of 18 and 30°C and photosynthetic photon flux densities of 700 and 325 μmol s^{-1} m^{-2} during a 9-h day period. Day/night aerial temperatures were 26/22°C.



agreement between predictions by the model and measured performance of two species of plants to perturbations in temperature and radiation indicates that the model structure is relevant to actual growth and can serve as a general model for growth of whole plants.

SUMMARY

The role of the mathematical model is to relate the individual processes to environmental conditions and the behavior of the whole plant. Using the controlled-environment facilities of the phytotron at North Carolina State University for experimentation at the whole-plant level and methods for handling complex models, we have developed a plant growth model to describe the relationships between hierarchial levels of the crop production system. The fundamental processes that are considered are (a) interception of photosynthetically active radiation by leaves, (b) absorption of photosynthetically active radiation, (c) photosynthetic transformation of absorbed radiation into chemical energy of carbon bonding in solube carbohydrates in the leaves, (d) translocation between carbohydrate pools in leaves, stems, and roots, (e) flow of energy from carbohydrate pools for respiration, (f) flow from carbohydrate pools for growth, and (g) aging of tissues. These processes are described at the level of organ structure and of elementary function processes. The driving variables of incident photosynthetically active radiation and ambient temperature as inputs pertain to characterization at the whole-plant level. The output of the model is accumulated dry matter partitioned among leaves, stems, and roots; thus, the elementary processes clearly operate under the constraints of the plant structure which is itself the output of the model. The values of those parameters,

such as rate constants for respiration, growth, etc., that cannot be directly measured as they operate in the intact plant but are necessary for mathematical description of the elementary processes are inferred from estimation procedures based solely on observations at the whole-plant level under the controlled and repeatable experimental conditions of the phytotron. The structure of the model thus provides the capacity for interpolating among the levels within a crop production system of a CELSS. Continuing development is being directed toward including nitrogen nutrition and carbon dioxide as inputs to the model, extending the structure of the model to include reproductive growth, and adapting the model to run on a micro-computer.

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CELSS INVESTIGATORS MEETING

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OPENING REMARKS

Sherwood Chang

Chief, Planetary Biology Branch, NASA-Ames Research Center

The biogenic elements that are involved in all living systems, is of equal importance to CELSS researchers as to those of us interested in planetary biology. Among the research thrusts encompassed by what we call exobiology, or planetary biology, is an understanding of the pathways by which these biogenic elements have made their way from their nucleosynthesis in stars to their incorporation into the compounds that were ultimately spawned on the primitive Earth before living systems appeared, and were the precursors of living systems.

Recent work on carbonaceous meteorites has uncovered compelling evidence that, indeed, there is material which has made its way to the Earth from the stars, and gives credence to the idea that early on similar materials played a very important role in the initial endowment of the primordial components of the plants from which living systems eventually emerged. Just as the history of these biogenic elements can be traced back to stars and their origins in nucleosynthesis, so I believe will the future of Earth's life be ultimately in the stars. And a journey to the stars means that humankind will be living in space, an idea that goes far beyond the idea simply of humans travelling into space for short trips.

The notion of humans living in space for lengthy periods is closely linked with discussions of Space Stations, Lunar Bases and future expeditions to Mars. Living in space brings to mind all these activities which lend quality to mere existence, and will probably occur in our grandchildren's lifetimes. And living in space is intimately linked with the growing of food in space. In the early history of this country, the farmer followed closely on the heels of the explorer, and it is quite conceivable that in the future exploration of space and settlement that the farmer will be there, too. The first steps may have us living on Space Station, or the moon, or the most exciting place of all, on Mars.

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PLANT GROWTH CHAMBERS

Dr. James H. Bredt, Chairman

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N86-19915

PLAN FOR CELSS TEST BED PROJECT

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Introduction

The concept of a Controlled Environment Life Support System (CELSS) is not new, and the survival of man in space has been demonstrated for 9 months by the Soviet Union. Most available data indicate that the problems with the long-term presence of man in space are those associated more with life support systems and less with the mechanical integrity engineered into the vehicles per se. As the duration of missions in space becomes longer and longer -- many months to years -- the need for constructing an integrated reliable life support system incorporating regeneration becomes increasingly crucial.

In order to develop a functioning CELSS, as represented in figure 1, test modules must be designed, constructed, integrated, and tested for performance and reliability. Such tests should be run in parallel with current basic research efforts. These tests will translate laboratory results into a chamber facility that integrates the essentials of plant growth and material recycling processes to show that it can be done on a practical scale. This effort is to be followed by a more sophisticated system that will accomplish manned tests of the CELSS as a life support system. Concurrently, space station experiments will determine whether there are fundamental problems with growing plants in weightlessness, and solve them or develop workarounds. Following the manned test of a ground-based engineering prototype, a space system will be developed, and its gravity-sensitive components tested on the space station as appropriate. That phase will be completed about 2000 A.D., so that engineers defining the 21st Century missions can work with hard data, and the design and development of CELSS systems for these missions can begin when required.

Four major components and two development areas are recognized as vital to the creation of a bioregenerative life support system:

COMPONENTS
1. Atmospheric regeneration
2. Food production
3. Food processing
4. Waste management

DEVELOPMENT AREAS
1. Engineering and control technologies
2. Analytical and monitoring capability

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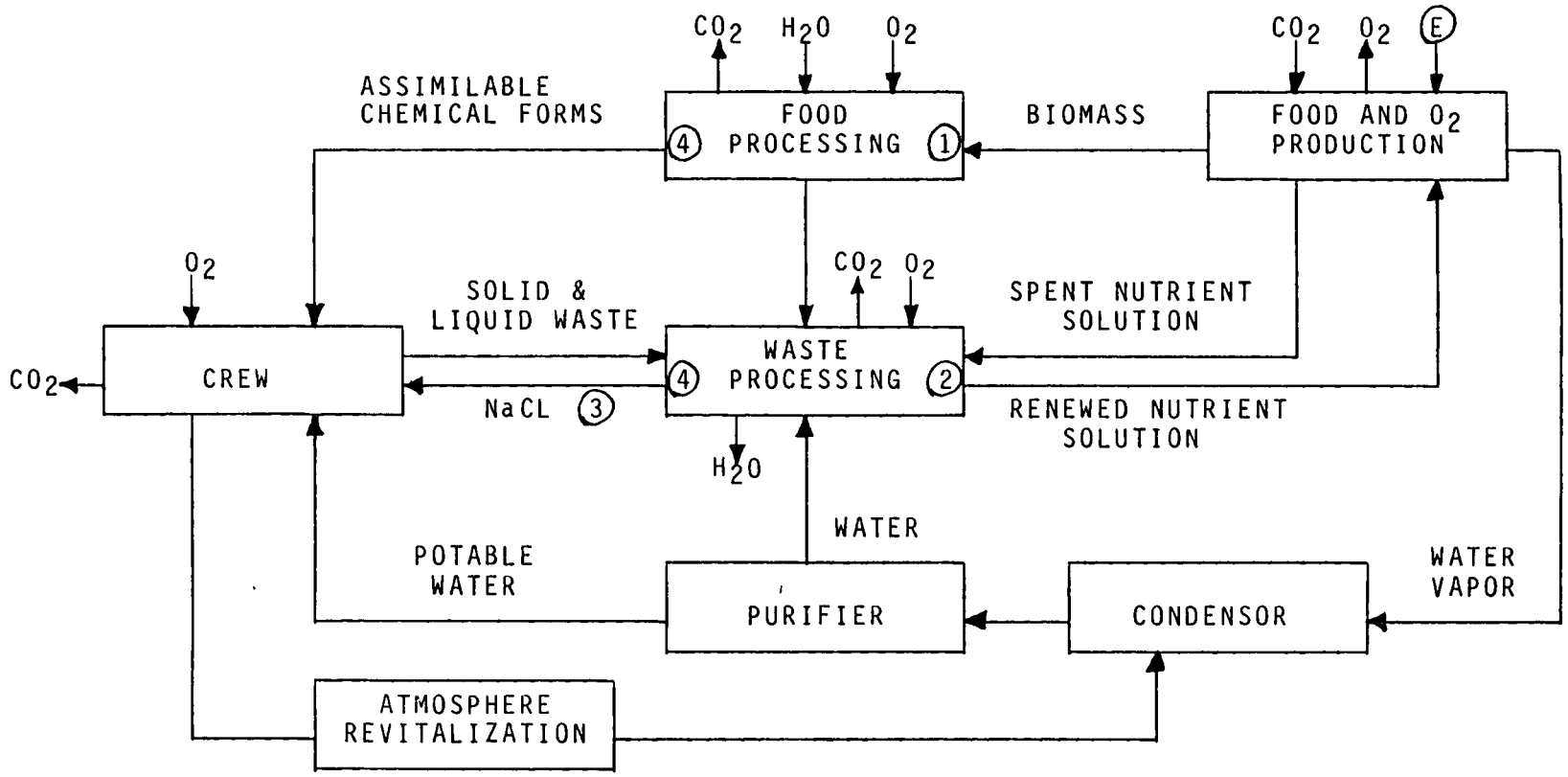
Kennedy Space Center

CELSS PROGRAM PROCESS FLOW DIAGRAM



Research Emphasis During FY 85 and 86

1. Biomass Utilization
2. Nitrogen Management
3. Salt Partitioning
4. Subsystem Definition

FIGURE 1

The entire range of waste management options for reducing waste must be examined critically. The regeneration of waste water into a useable product will be a high priority in this project. Regardless of which biological system creates the reduced carbon ultimately destined for human consumption, processing will be essential. Current food processing techniques must be adapted, streamlined, miniaturized and effectively tailored to the needs of space. It is highly probable that a coupling of physical, chemical, and biological techniques for the treatment and segregation of waste and the efficient conversion of biomass to an edible substance will be the ultimate solution to the generation of a CELSS. An objective is to convert waste to useable foods, fuels, or other needed commodities such as atmospheric supplements.

Atmospheric regeneration must concentrate on maintaining a proper CO_2/O_2 balance under closed chamber conditions. Monitoring for the build-up of trace contaminants will be another major concern in researching this program component. Development of food production hardware will be closely coordinated with the atmospheric regenerative component. Food production can be accomplished with higher plants and/or photosynthetic microorganisms. Although not totally excluded, any animal component in food production during early phases of this project will be very limited. The growth of botanical biomass of dietary satisfaction to man, coupled with appropriate atmospheric regeneration, will be the principal objective of this project.

General: Project Technical Plan

The CELSS testbed project will achieve two major goals:

1. It will develop the knowledge and technology needed to build and test biological or combined biological physico-chemical regenerative life support systems.

2. It will fabricate, test, and operate ground based facilities to accomplish proof-of-concept testing and evaluation leading to flight experimentation.

The project will combine basic research and applied research/engineering to achieve a phased, integrated development of hardware, systems, and techniques for food and oxygen production, food processing, and waste processing in closed systems as follows:

1. Design, fabricate, and operate within three years a botanical production system scaled to a sufficient size to verify oxygen and nutrient load production (carbohydrates, fats, proteins) at a useable level.

CELSS PROJECT

MASTER SCHEDULE

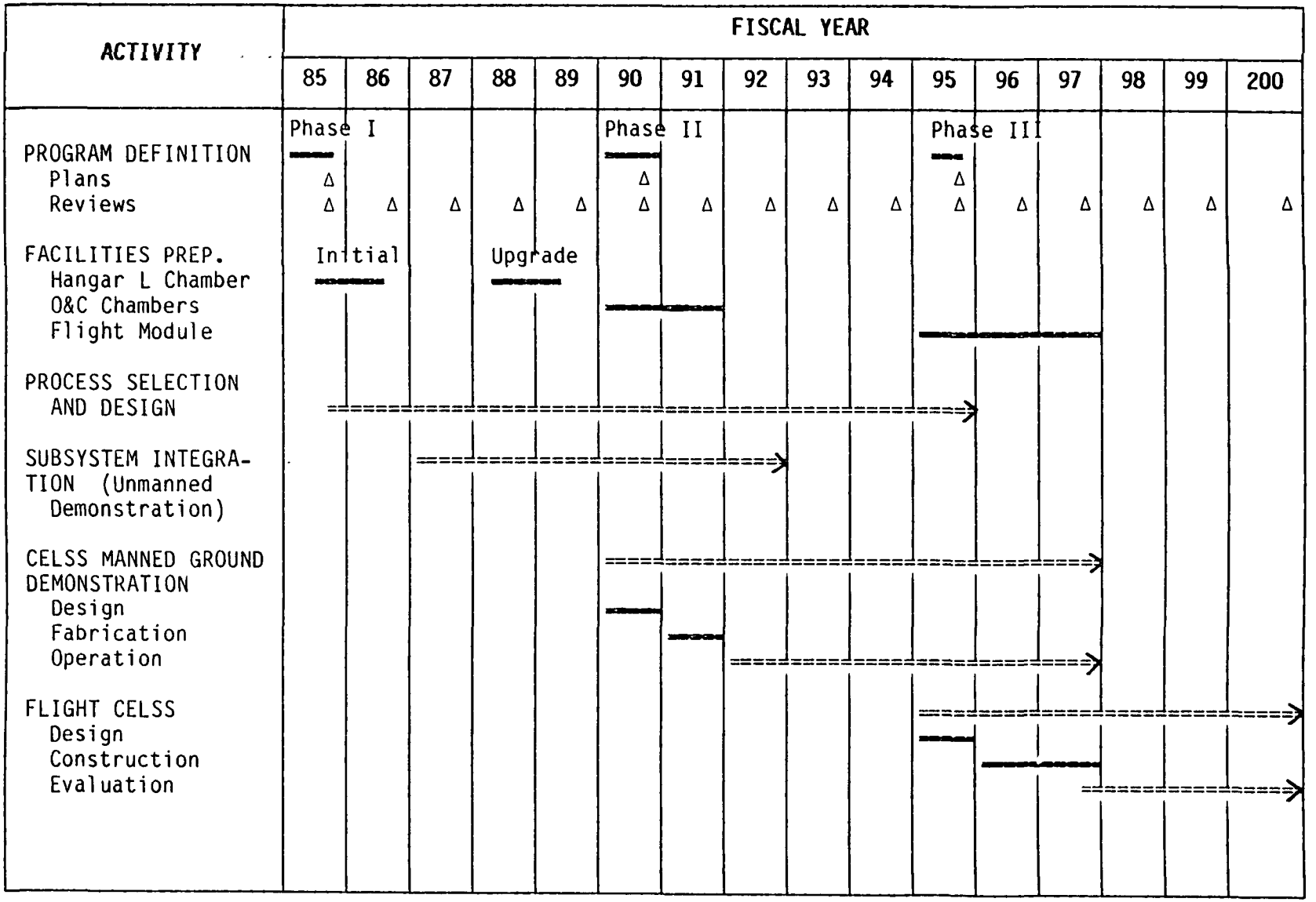


FIGURE 2

2. Develop within five years a waste management system compatible with the botanical production system and a food processing system that converts available biomass into edible products.

3. Design, construct, and operate within ten years a ground based candidate CELSS that includes man as an active participant in the system.

4. Design a flight CELSS module within twelve years and construct and conduct initial flight tests within fifteen years.

The testbed project will be divided into three phases with each phase conducted in series (Figure 2). The successful completion of one phase will be required before proceeding to the next. Each of the phases will be sufficiently distinct to produce important useful results even if the project is terminated prior to initiation of the subsequent phase. The three phases are:

1. Bioregenerative System Evaluation Test
2. Manned CELSS System Evaluation Test
3. FLight System Evaluation Test

Each of the three phases will take from 3-5 years to complete. A flight unit for testing in space will be completed within fifteen years.

This plan will be revised near the end of Phase I to review detailed objectives and approach to Phase II, and again during Phase II for Phase III.

Phase I: Bioregenerative Systems Evaluation Test, 1985-1990

The specific objectives of this portion of the program are:

1. Develop a closed chamber facility as a test bed for evaluating bioregenerative systems under ground based conditions.

2. Design and develop a higher plant production module and optimize it's efficiency for recycling and energy use. Incorporate microorganism production systems into the module as appropriate.

3. Operate the plant production module as a closed system for at least a six month period of time.

4. Determine the current status of food processing and water management systems and identify candidate systems for testing with the plant production module.

HYPOBARIC CHAMBER

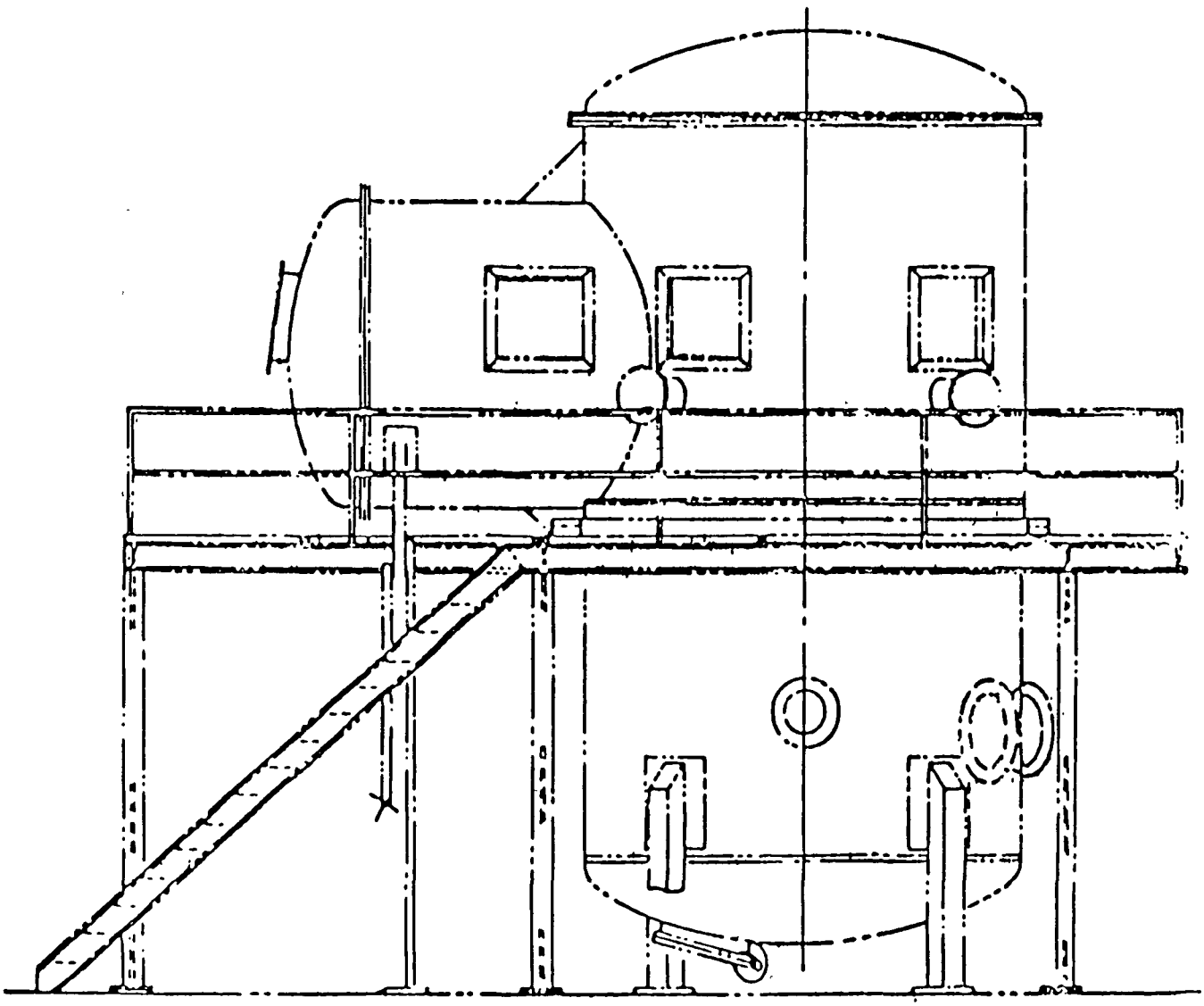


FIGURE 3

5. Develop selected food processing and waste management modules and test them as a part of an integrated system with the plant production module.

6. Design an integrated ground based CELSS with man as an integral part of the test facility.

This phase of the program will utilize a 6.7m x 3.6m cylindrical closed chamber facility in Hangar L. (figure 3). This hypoboric chamber (from the Mercury Program) will be modified to accomodate plant growth. Using this chamber, a plant production system will be developed and tested. Tests will be conducted with candidate higher plant species, concentrating on a single species community and later multiple species communities. Emphasis will be placed on engineering improvements during the tests, primarily concerning nutrient delivery systems, automated maintenance and harvest systems, lighting elements, and monitoring and control instrumentation. Chamber operations and systems integration will be accomplished through a NASA/Contractor team onsite at the Kennedy Space Center. The chamber will be refurbished and ready for testing by March, 1986 and plant studies will begin at that time. An AIBS advisory panel has been appointed to oversee this portion of the project.

A set of chamber tests will be initiated in April 1986 to establish the operational capability of the plant production systems and baseline mass and energy flux for the production module. Microorganism production systems will be developed and incorporated into the chamber tests as required. At least one extended time period ($\geq$ 30 days) test with multiple species crop community will be conducted during the first 2 years of the project.

As problems develop during chamber tests, decisions will be made to whether they can best be researched in the chamber or should be referred to other laboratories. Contracts will be developed with outside organizations on a specific problem solving basis as the test program dictates. Anticipated areas of research include definitive nutrient requirements, selected crop production, evaluating plant propogation techniques, plant monitoring instrumentation, microorganism culture systems, lighting systems, and higher plant nutrient delivery systems. Actual areas of research will be identified during the second year of the project and continuing for the remainder of Phase I. New concepts and equipment resulting from this research will be incorporated into the test facility as appropriate to improve the system starting with year 3.

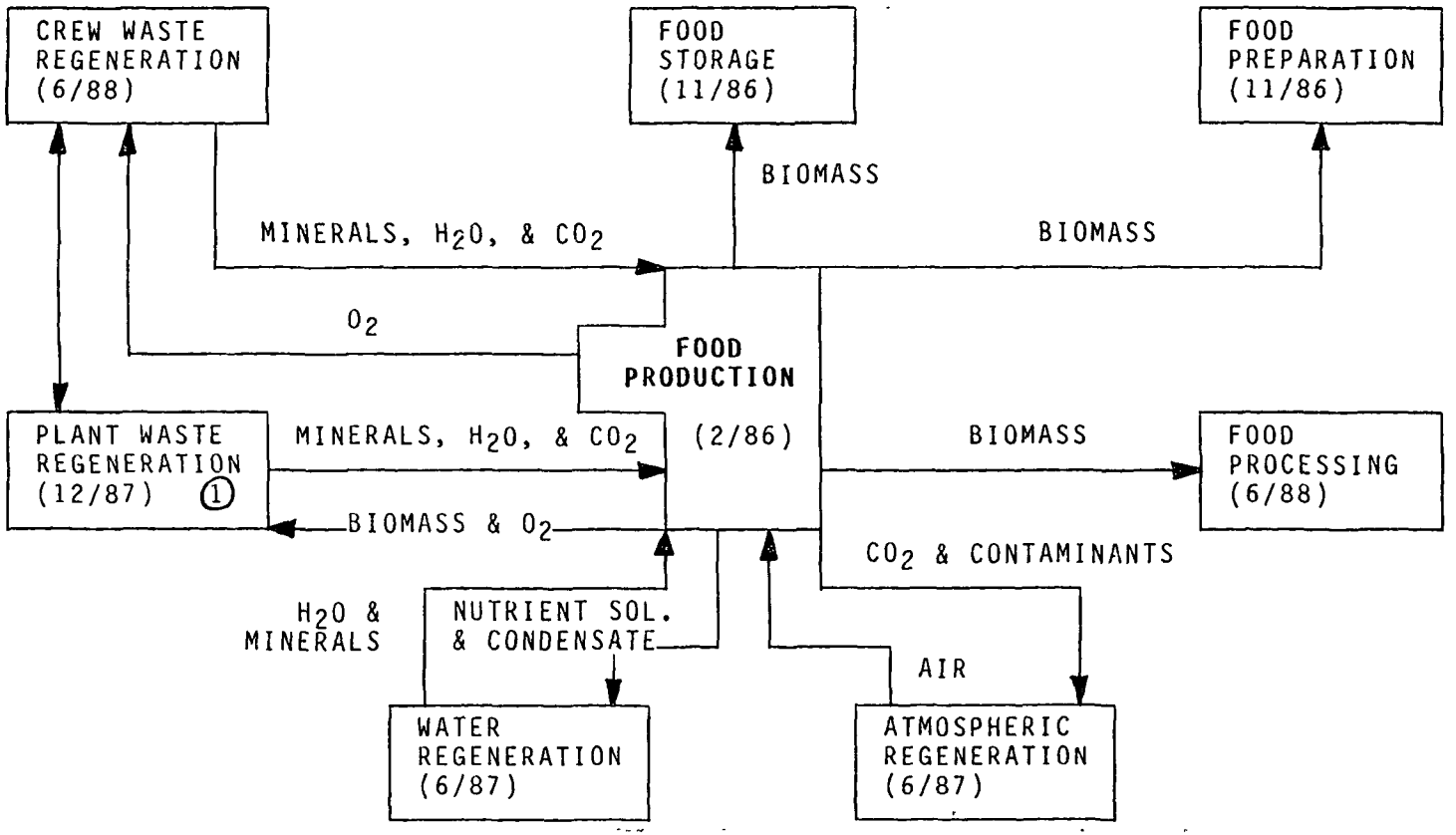
A functioning plant production system will evolve over this 3-5 year Phase I portion of the program. Tests will become longer in duration reaching a $\geq$ 90 day period by the end of this phase. An efficient, highly automated plant production module will be a product of this Phase I effort. The integration of food processing and waste management systems will also be done during Phase I. Food processing and waste management systems will be assessed while the food production module is being developed, and the best systems selected for evaluation. Food processing will concentrate on minimizing and converting plant wastes into reuseable components, and waste mangement will emphasize nitrogen cycling. This follow-on system selection will be accomplished with guidance from AIBS technical committees that will meet for the first time in August 1985. A revision to the project plan will be made by the end of 1985 that addresses in detail how the research and development of these systems will proceed.

The general approach to how the atmospheric control, waste management, and food processing components will be developed, integrated, and evaluated can be outlined. Systems to control gaseous contaminants in the atmosphere of the closed plant production facility will be the first addition to the chamber. It is anticipated that volatile hydrocarbons will be the major contaminants resulting from natural metabolic processes (e.g., methane, ethylene) and from outgassing of materials and equipment (e.g., thalates). Systems developed previously have included cyrogenic traps, incinerators, and carbon adsorption. Contaminant control systems must be interactive with atmospheric control systems. Exact requirements must depend on information gained from detailed analytical chemical studies of processes, materials, and equipment early in the operation of the plant procduction chamber. An atmospheric "finetuning" system will be added to the air handling ducts of the chamber during 1986.

A water recovery system will be integrated into the test that will recycle spent nutrient solutions and condensate from the chamber. The system will be derived from appropriate filtration and/or chemical treatment techniques. The ultimate objective of this system will be to improve the generation of water which is useful in sustaining plant growth in a timely and efficient manner. The water recovery system will be integrated with the chamber facility during late 1986.

Integration of a candidate waste management system with the plant production unit will be completed by late 1987. This system will be operated in laboratories adjacent to the chamber using primarily nonedible plant biomass. Human wastes could be added to the system later as appropriate to develop satisfactory operations. Much of the plant waste product could be recycled through the solid waste management system involving biodigesters, pyrolyzers, and/or incinerators. Unfortunately,

CELSS PROGRAM
COMPONENT INTEGRATION



① – Concentrate initially on nitrogen cycling and conversion into food

MONITORING AND CONTROL:
1. CO_2 and O_2
2. O_2 trace contaminants and viable nonedible particulates
3. Pressure, temp., humidity
4. Biomass (carbohydrates, proteins, lipids, & vitamins)
5. Water
6. Major nutrients
7. Energy (ie. electricity)

FIGURE 4

much of the engineering effort relative to these systems has centered on disposal rather than the recovery of water and chemicals. Additional information will be needed to choose and evaluate the optimum process once the most likely solid and liquid wastes are known. Design parameters are available for many physiochemical and biological solid organic waste treatment processes, but integrated, operational systems have not been completed. In addition, questions on mineral separation, recovery and conversion, vitamin recovery (or supplement), plus information on sanitation, safety, recycling time, and system by-products must be researched.

Food processing systems also will be developed in laboratories adjacent to the plant production chamber. Preparation and storage of edible food materials will be one part of this system. The more difficult part of this system will be that portion designed to convert nonedible plant biomass into an edible material. Candidate techniques to accomplish this conversion include microbial, chemical, and/or enzymatic systems. The development of food processing systems will begin in early 1986, and chamber tests will begin in late 1987 or early 1988. It should be stated perhaps, that plants selected for use in the system will be chosen, in part, for their high percentage of edible mass, thus minimizing this problem on the input side. Uneaten residue may, however, neutralize this hoped for advantage.

All candidate waste management and food processing systems will be interfaced with the plant production chamber facility and operated, tested, and evaluated. Tests and research will emphasize the integration of multiple subsystems into a total operational system. Measurements made during chamber tests will include CO_2 uptake and O_2 generation, moisture levels, nutrient uptake, nitrogen concentration, waste decomposition, and plant growth and productivity. Special attention will be given to the generation of contaminants under closed atmosphere and recirculating nutrient solution conditions. Improvement in the efficiency and reliability of the integrated systems to operate as a bioregenerative component will be the primary goal of this phase of the project. Tests will concentrate on mass and energy fluxes, and systems to improve recycling.

The final product of Phase I will be an integrated system that will produce food (major nutrients), produce O_2 and consume CO_2, and recycle essential waste and water components sufficient to maintain 3-4 people (figure 4). The goal is to make the system functional at a 4-5 KW maximum power level and 10 M^2 growing area per person. Phase II and III will follow in series after the completion of Phase I. General objectives for these later two phases have been established, but details of technical plans are scarce and, therefore, will not be discussed in this report.

N86-19916

PLANT GROWTH CHAMBER 'M' DESIGN

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INTRODUCTION:

Crop production is just one of the many processes involved in establishing long term survival of man in space. The benefits of integrating higher plants into the overall plan was recognized early by NASA through the Controlled Environment Life Support System (CELSS) program. As it continues to explore the fundamental concepts of plant life, plans are to develop the capability for integrating the biological, physical, chemical and control components into a working ground-based unit.

The first step in a sequence of activities planned for the John F. Kennedy Space Center is to design, construct, and operate a sealed (gas, liquid, and solid) plant growth chamber. A 3.6m diameter by 6.7m high closed cylinder (previously used as a hypobaric vessel during the Mercury program) is being modified for this purpose. The chamber is mounted on legs with the central axis vertical. Entrance to the chamber is through an airlock. It is located in Hangar L on the Cape Canaveral Air Force Station. This chamber will be

devoted entirely to higher plant experimentation. Any waste treatment, food processing or product storage studies will be carried on outside of this chamber.

Its primary purpose is to provide input and output data on solids, liquids, and gases for single crop species and multiple species production using different nutrient delivery systems.

INTEGRATION PLAN

The food production component will become the central focus for the food processing, food preparation, solids and liquids waste treatment, gas regeneration, storage, and monitoring and control components. Data resulting from this food production unit will be used in the design and operation of other components. This chamber is expected to be operational in March 1986.

CHAMBER DESIGN

Physical Features. The chamber is 3.6m (12 ft.) diameter by 6.7m (22 ft.) high containing 68m^3 (2500 ft^3). Approximately 25m^2 (250 ft^2) of conventional crop growing area can be obtained by dividing the chamber into 2 sections with 2 grow-

ing levels each, as shown in Figure 1. This chamber should produce enough edible dry matter to supply one-half of the diet for a person based on a production rate of $10g/m^2/day$ (Tibbitts and Alford, 1982) and a consumption of 500g of edible dry matter per day. A solid floor between levels will permit different radiation treatments to be conducted even though all of the other parameters will not be affected. Crops can be grown around the periphery of the chamber leaving the center clear for work, a lift and a ladder.

Temperature and moisture regulation for the growing area and for the lamp banks will take place outside the chamber. The chamber is to operate at .5 kPa (2 in H_2O) pressure and chamber air will to be circulated through absolute filters. Carbon dioxide will be from bottled gas and air will be exchanged with the atmosphere.

To accommodate tall crops the lower lamp banks on each level will be removable. All crop support shelves will be adjustable from the lower position to within .56m of the lamp bank. The walls will be finished with mirrored surfaces.

Safety measures will be designed and built into the chamber to take care of equipment failure and human accident. Television cameras will monitor both levels.

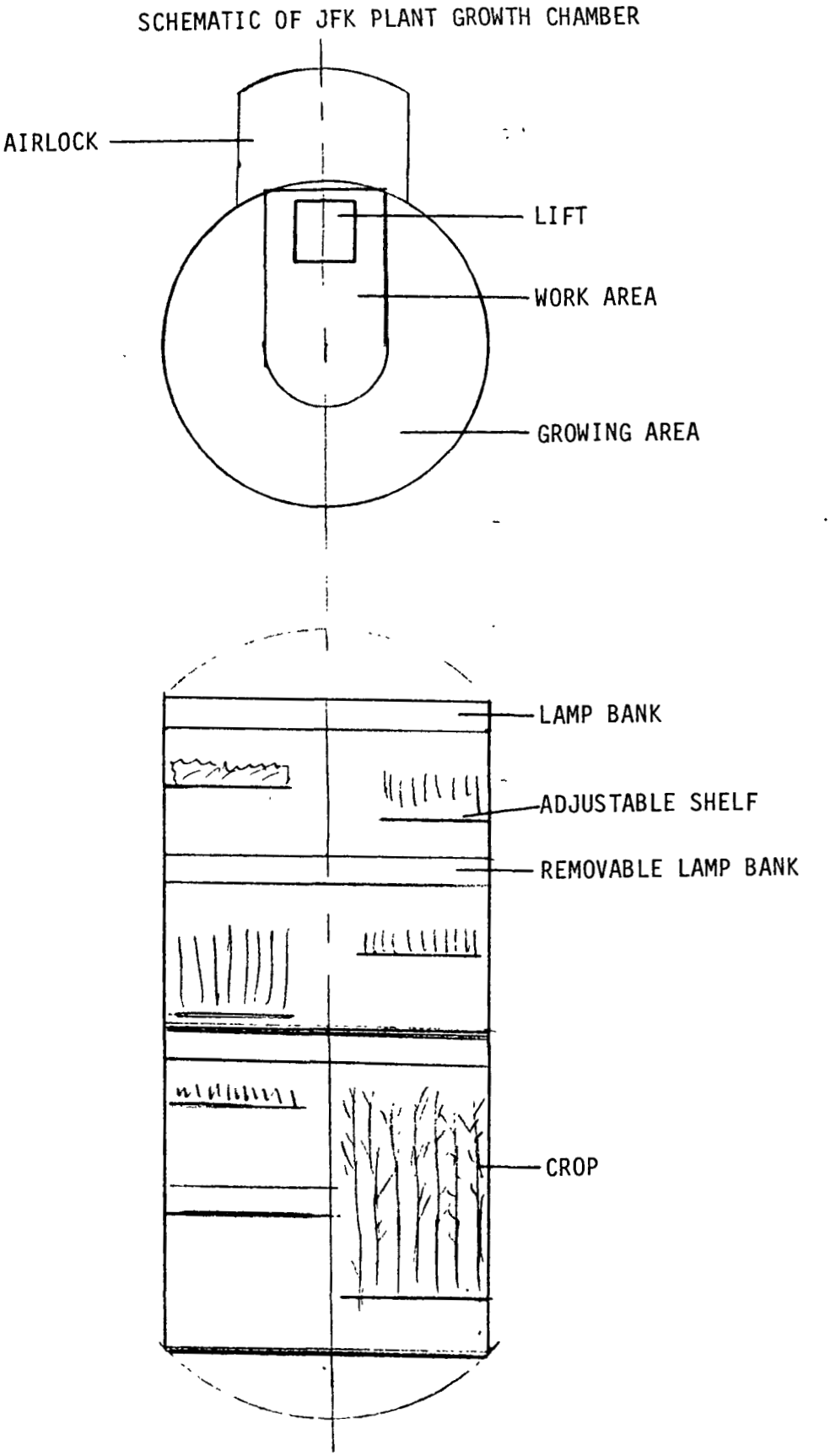
SCHEMATIC OF JFK PLANT GROWTH CHAMBER
AIRLOCK
LIFT
WORK AREA
GROWING AREA
LAMP BANK
ADJUSTABLE SHELF
REMOVABLE LAMP BANK
CROP

FIGURE 1

Growth Parameters. Because of the need to grow many different crops, the limits on essential growth parameters were set as shown in Table 1. The limits will permit a wide spectrum of experiments to be conducted. It will be possible to maintain two light regimes on the two levels of the chamber. Temperature, moisture, and carbon dioxide will be uniform throughout the chamber. Nutrient solution composition, concentration, pH and temperature can be specified as can nutrient quantity per plant, and nutrient velocity past the plant root when using a film.

Nutrient Delivery System. A conventional nutrient delivery system will be used for the initial plant growth experiments. It will be flexible allowing for type of growing systems (trough or pipe, gulley, and tube) and modes of production (continuous and all-in/all-out). Specific requirements for the particular crop and experiment can be met.

Experimental nutrient delivery systems such as the Capillary Effect Root Environmental System (CERES) (Wright and Bausch, 1984) will be studied. Crop production will be used as a part of the overall evaluation on nutrient delivery systems.

Table 1. Environmental parameters to be monitored and controlled in the plant production compartment.

PARAMETER	LIMIT LOW	LIMIT HIGH	Max. CONTROL ERROR	Min. MONITOR SENSITIVITY
Photosynthetically-active radiation at plant level ($\mu mol \cdot s^{-1} \cdot m^{-2}$)	200	$\geq$1000	NA	NA
Photoperiod (min)	species-specific	continuous	1	NA
Temperature (C)				
light	15	40	1	0.2
dark	10	30	1	0.2
Humidity (%RH)	60	$\geq$90	7	2
Carbon dioxide (ppm)	300	$\geq$2500	10	10
Oxygen (ppt) (added or scrubbed)	control to $\leq$20.9%		10	5
Air movement across leaf canopy ($m \cdot s^{-1}$)	2	5	NA	0.1

MONITORING AND CONTROL

It is necessary for this chamber to furnish data on energy, solids, liquids, and gases. Inputs and outputs of solids and liquids for the various experiments can be obtained.

Continuous monitoring of oxygen and carbon dioxide will insure that crop growing conditions are maintained. Periodic sampling of ethylene and other gases (Table 2) will provide information on specific crops, multiple crop species, and processes needed for future studies.

Initially, service personnel will wear face masks that are supplied with outside air. Later in the program, and as experiments dictate, complete suits may be worn by personnel entering the chamber which should permit a complete thermal, moisture, and gas analysis.

EXPERIMENTS

It is imperative that performance of the chamber be evaluated with a single crop species. Bush beans have been suggested as a good candidate for this first crop. Other crop species, modes of operation, and nutrient delivery systems can be added as confidence is gained with analysis of the system operation.

Table 2. Gases and particulates that should be controlled and/or monitored in the test chamber.

GAS	EXPECTED CONC.	MIN. INSTR. SENS.
Oxygen	20.9%	0.1%
Nitrogen	78.1%	0.1%
Carbon dioxide	300 to $\geq$ 2500 ppm	10 ppm
NO		
NO_2		
N_2O		
Ozone		
CO		
Ethylene		
H_2S		
Neon		
Ammonia		
Chlorine		
Fluorine		
Formaldehyde		
Methane		
Propylene		
Propane		
Vinyl Chloride		
Terpenoids and oils		
Krypton		
Freon		
(others as discovered)		
Pollen		
Mold Spores		
Dust		

Experiments designed to maximize volume utilization, minimize energy requirements, and minimize water useage are important. Others to determine the initiation points of toxic substances (solid, liquid, and gas) will be studied if needed. Studies to evaluate shoot-to-root, and edible-to-non-edible ratios need to be conducted. A careful assessment of food and oxygen production per unit of area and per unit of volume for several crop generations must be made. These data will provide a base for further research and for a more deliberate design of future food production components.

This chamber will contribute toward the detailed understanding of water (condensate, transpired, nutrient solution, and plant) recycling, biomass treatment, and gas regeneration. It will become the test-bed for controlled processes leading to recycling of solids, liquids, and gases.

Crops destined for study and evaluation include: beans (bush), wheat, potatoes, soybeans, rice, carrots, cowpea, winged bean, etc. These are some of the crops listed by Tibbitts and Alford, 1982.

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N86-19917

OPERATIONAL DEVELOPMENT OF SMALL PLANT GROWTH SYSTEMS

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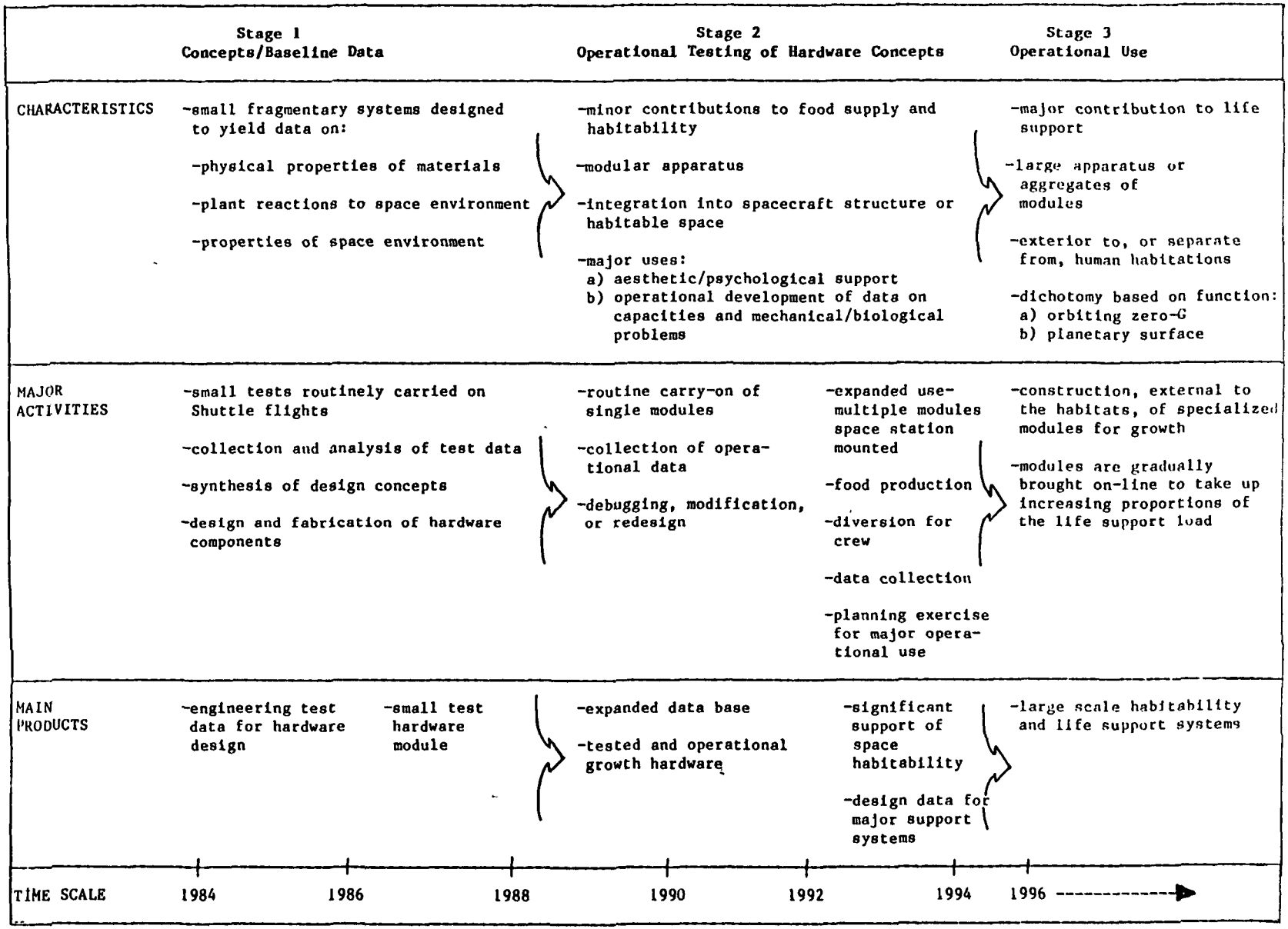
Background

This report summarizes the results of a study undertaken on the first phase of an empirical effort in the development of small plant growth chambers for production of salad type vegetables on Space Shuttle or Space Station. The overall effort is visualized as providing the underpinning of practical experience in handling of plant systems in space which will provide major support for future efforts in planning, design, and construction of plant-based (phytomechanical) systems for support of human habitation in space. The assumptions underlying the effort hold that large scale phytomechanical habitability support systems for future space stations must evolve from the simple to the complex. The highly complex final systems will be developed from the accumulated experience and data gathered from repetitive tests and trials of fragments or subsystems of the whole in an operational mode. These developing system components will, meanwhile, serve a useful operational function in providing psychological

Figure 1. Time course for development of space-borne habitability support systems.



support and diversion for the crews, and/or some modest contribution to the food supply.

Rationale

An empirical approach appears entirely justified based upon our quite limited knowledge of the space flight environment and the responses of plants in that environment. It is useful to remember that the basis of what we consider to be modern terrestrial agriculture and horticulture was laid over the centuries in empiricism and art. Modern science and engineering have produced some remarkable advances, but none of these would have been possible without the ability to build upon the ancient foundation. That same foundation of experience is not yet available to those who wish to culture plants in space. We, therefore, will only be able to make appreciable progress if we have some reasonable body of empirically derived data upon which to build.

A general scenario for the long-term development of plant culture systems for space is presented in Figure 1. There is nothing about the target system or the pathway to its development that specifies precise configurations or technologies employed. We are, in effect, deferring specific questions related to the selection of final system concepts and approaches until we have gained sufficient data and operational experience in the handling of plants in space to support rational decisions. The present report summarizes the

results of efforts in definition of plant growth systems which will provide the needed operational experience and data on handling plant systems in space as well as some practical support of the flight food system.

The focus of this effort has been upon, as indicated in the Introduction, the examination of the practical problems of in-flight plant growth systems at the simplest useful level. It began with a given set of constraints and requirements and explored the possibilities within the envelope of these requirements. A relatively large number of dead-ends were encountered and while these are useful to know, a detailed account will largely detract from a discussion of the concept development. The discussions and diagrams which follow outline the major steps in the process of developing approaches to small plant growth systems for Shuttle.

Design Criteria

The following constraints were placed on the plant growth system:

A. Functional Requirements

1. to provide useful contributions to the food system - prime requirement;
2. to test empirically the "best guess" of what a growth system should be;
3. to provide a test bed for acquisition of experience and data.

B. Hardware Configuration

1. must fit into a standard slot in the orbiter; the bulkhead storage locker system;
2. must have simple, low cost construction;
3. must use the least complex growth systems consistent with adequate function; and
4. must be configured to grow salad-type vegetable plants.

After an examination of the various possibilities within the constraint envelope, three general approaches were adopted and pursued. The first, and simplest, was in the use of seed sprouts as a low cost, low technology means of producing fresh salad vegetable material. The second was, more conventionally, the use of standard green vegetable plants in a small, lighted growth chamber. The third was a test bed configuration for acquisition of basic data on plant response in zero-G.

Sprouting Systems

Seed sprouts offer a number of advantages both as a quick and easy way of providing fresh vegetable material in short Shuttle flights and as a more routine food for much longer duration space flights (Table 1). Seeds of various vegetables and field and forage crops can be stored dry for considerable periods of time. When fresh sprouts are required, water is the only input needed to bring about a five to seven-fold increase in fresh weight. The most important characteristic

Table 1. Comparative characteristics of seed sprouts and mature salad vegetable plants as candidates for testing and use in small, in-flight fresh food systems.

CHARACTERISTIC	SPROUTS	MATURE PLANTS
Time to Maturity	*4 - 6 days*	*Up to 90 days*
Complexity of Apparatus	Simple and compact: Necessity only for water and aeration; orientation not problematic.	More complex and larger: Provision for soil and nutrients, light and temperature control, orientation of plant parts.
Variety of Food Items	Limited: A single type of item with limitations in taste texture and range of uses.	Variety large: Limited only by ability to contain and grow the plant.
Popularity/Aesthetic Appeal	Limited because of food habits of general population. Not aesthetically appealing.	Wide popularity; high aesthetic appeal both during growth and at consumption.
Nutritional Value	Limited on amount which can be consumed raw without complications.	Adequate.
Processing/Use	Very simple. No waste, no mess.	Not complex, but with significant waste disposal problems.
Experimental Value	Useful for development of fluid/air handling and control technology.	Useful for development of soil, nutrient and microbiological technology. Useful for light and energy technology development and for study of air/gas handling.

is the marked increase in food value associated with sprouting (Table 2). Vitamin content increases dramatically, fat and carbohydrate content are reduced while relatively little protein is lost, fiber content increases, and many of the inhibitors and toxicants associated with seeds appear to be lost or reduced significantly.

	DRY SEEDS												SPROUTS									
Plant	Nutritional Value				Vitamins				Minerals				Nutritional Value				Vitamins				Minerals			
	Cal	Pro	Fib	Fat	A	B1	B2	C	P	K	Ca	Fe	Cal	Pro	Fib	Fat	A	B1	B2	C	P	K	Ca	Fe
Amaranth		16.7	4.2	7.5		.21	.09	2.25		3750	1701	93.4	246	24.0			6918	.54	1.08	546	456	2796	1818	26.4
Lentil	340	24.7	3.9	1.1	60	.37	.22	t	337	790	79	6.8	304	23.8		1.0		.50	.20	t	240	670	39	7.6
Mung	340	24.2	4.4	1.3	80	.38	.21	—	340	1028	118	7.7	245	26.6	4.9	1.4	140	.91	.91	133	448	1561	133	9.1
Soybean	403	34.1	4.9	17.1	80	1.1	.31	—	554	1677	226	8.4	276	37.2	4.8	8.4	480	1.4	1.20	78	402	—	288	6.0

Table 2. Nutritional value of dry seeds and their sprouts.

Apparatus necessary for seed sprouting is minimal. Light, soil, and the containers necessary for whole plant cultivation are not necessary; water and a well drained, aerated container are the major requirements. The space environment with the altered conditions of fluid movement places some constraints upon the process, but once recognized, elimination of these constraints is merely an engineering problem.

A number of potential issues were addressed and resolved during the development effort. These will only be listed here:

1. microbial contamination;
2. toxicant content of seeds and sprouts;
3. selection of species for use in flight conditions;
4. sources of water and water addition schedules particularly as they related to flight conditions; and
5. storage and/or pre-germination of seeds.

None of these were seen as having an appreciable impact upon the use of seed sprouts in Shuttle or extended missions.

The systems depicted in Figure 2 represent the end point of an exercise which considered several different approaches to the problem of routinely producing salad sprouts on Shuttle. It utilizes the storage locker and the configuration of the standard half-locker tray (NASA, 1984) as a structural envelope. A number of issues related to operation remain to be worked out; many will depend upon flight testing for resolution.

The general features of the systems are as follows:

1. The seed sprout container is the standard six ounce Shuttle food system pack. Seeds are packaged and stored dry under vacuum in the same manner as the dehydrated foods.
2. The dry packs are installed in the unit as needed.

The first system uses a tool which perforates the bottom of the food pack, and the flexible cover is either perforated or removed. The second system connects to the standard "drinking straw" of the food pack.

3. Water is added to the dry seeds to initiate germination and is added periodically, as required, to maintain sprouting. Watering could be accomplished by hand, but a system for sensing moisture content and adding water as needed could be utilized.

4. In operation at micro-G, the system uses either a fan to pull a low flow of air down through the seeds into the space below or a microprocessor controlled water and air metering system connected to the standard "drinking straw". In the first system, the small air flow serves to aerate the seeds and in micro-G, theoretically, should be adequate to prevent the seeds or sprouts from floating out into the cabin environment. In the second system, no opening to the cabin is permitted.

The configurations shown in Figure 2 have been built and operated on the ground as a nonflight-qualified items. Issues such as watering practice, air flow, and general workability of the apparatus in micro-G will only be resolved by flight experience.

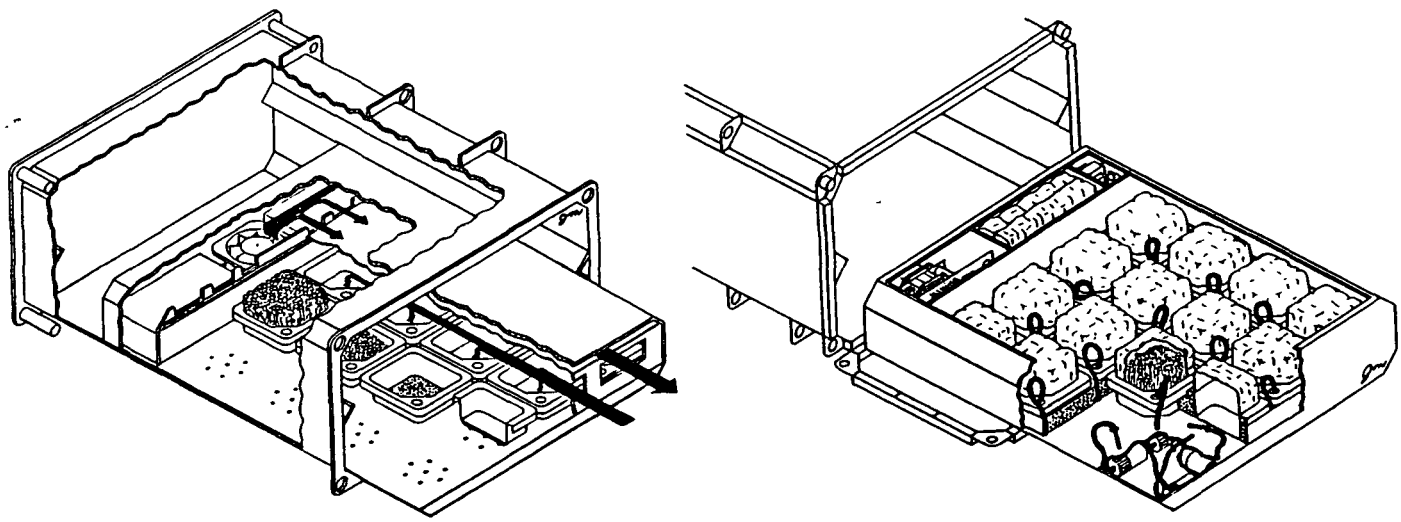


Figure 2. Configuration of Shuttle Middeck locker-based seed sprouting systems. Units are sized to a half-locker tray.

Whole Plant Chambers

The more conventional approach to growth has taken, as a starting point, the envelope of one middeck forward bulkhead locker, the exterior middeck dimensions of which are 21.062 in. x 10.757 in. x 18.125 in. A detailed description of the locker is included in the Orbiter Middeck Payload Provisions Handbook (Hix, 1984). Because of the practical approach taken in this effort, many of the orientation and space constraints of an earlier effort (Maine et al., 1979; Cowles et al., 1982, 1984) do not apply and thus more optimal use could be made of the available space.

The general effort had two thrusts:

1. A study of optimized configuration for the envisioned use; and
2. Consideration of the general array of technology to be taken into account in development of a growth system.

Figure 3 schematically summarizes the various issues as outlined below:

1. Optimal configuration of the container.
   a. Geometry - which may be very dependent upon tests in a zero-G environment.
   b. Volume of contained area - related to plant size and species.
2. Composition of the growth/support medium.
   a. Synthetic, versus natural materials, versus a modified hydroponic/aeroponic system.
   b. Porosity and affinity for water.
   c. Fertilizer delivery system -slow release, versus ion exchange, versus hydroponic solution.
3. Operating parameters.
   a. Air and liquid movement rates.
   b. Temperature regulation of the root zone.
   c. The role of microorganisms - important because of disease, human and plant, but also because microbes could function in atmosphere scrubbing.
4. Mechanical systems.
   a. Air and water handling, zero-G separation of the two being the main problem.

b. Water cleanup and conditioning.

i. Nutrient adjustment.

.. Removal of root and microbial metabolites.

5. The adaptability of various plant species to the system.

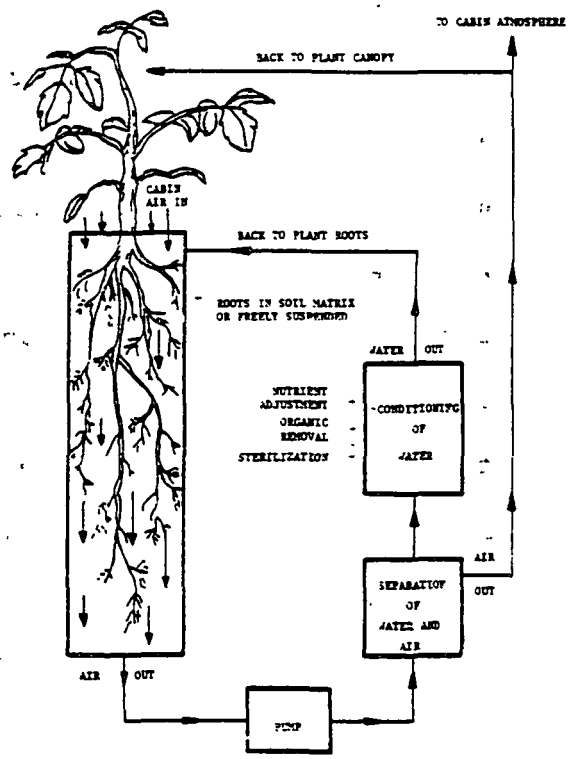


Figure 3. Concepts for control of watering and aeration in a zero-gravity environment.

All of the points listed are subjects of continuing efforts. This report and the growth chamber concepts it presents are merely single frozen moments in an evolving field. Much of what we add will depend on flight test data and experience.

Growth Chamber Concepts

The growth chambers, shown in Figure 4, embodies most of

the issues listed above. Figure 4-b depicts a configuration appropriate for dwarf varieties of small, bush-type plants such as tomatoes or peppers. Figure 4-a depicts the configuration more appropriate for a low profile leaf or root vegetables such as lettuce, onions, or radishes. In all tests it has been used to grow "feldsalat" style lettuce. All exterior dimensions of the chambers shown are the dimensions of the Shuttle locker. Materials are yet to be determined by flight configuration. In the models depicted, all materials are off-the-shelf foamcore, plexiglas or lexan for the shells with standard fluorescent light and electronic components. Units currently under construction utilize light metal and plastic sized to fit inside a standard locker which provides the primary structural strength and containment. Air inlets and outlets, power cables, and control panels are mounted in the modified locker access door provided as an alternative to the standard locker door (Hix, 1984).

Air flow is set to move across the plant from the Shuttle environment and to exit across the lamps to provide cooling. Growth media and roots are aerated and water is controlled by positive movement of air down through the growth substrate area aided by a small vacuum pump. Water is metered into the growth substrate area under control of a microprocessor controlled system that limits overwatering and movement of excess fluid.

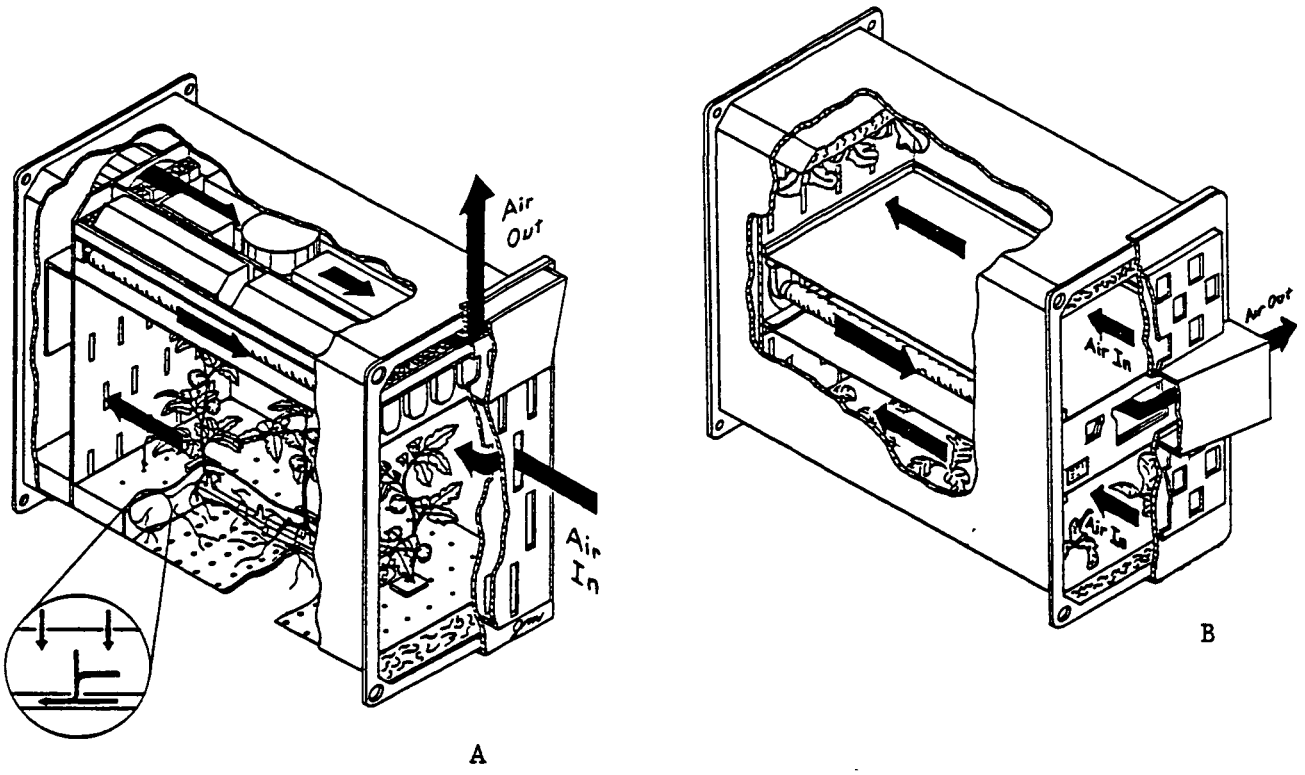


Figure 4. Configuration of shuttle middeck locker based growth chambers for salad vegetables. A - Dwarf fruit bearing plants. B - Leaf and root vegetables.

Working models of both configurations have been built and tested in the 1-G configuration with orientation of the lights, and other components, 90° to the flight orientation as the instruments would be mounted in a Shuttle locker. These configurations thus form a baseline and starting point for an effort aimed at flight development and testing of small growth systems.

Test Bed Configuration

Thus far two different plant growth chamber designs have flown on Shuttle. These instruments were essentially single

purpose, built to test specific hypotheses in gravitational biology. Published results from these experiments show in agreement with a number of reports from the Soviet experiments, that root growth had been affected in ways that were visible at the microscopic level (Cowles et al., 1982, 1984; Krikorian and O'Connor, 1982, 1984; Slocum et al., 1982, 1984). This provides some circumstantial evidence to support the intuitive expectations -- which are based on ample experimental evidence at one-G (Gates, 1975) -- that absence of gravity-driven convection will have a considerable impact upon the normal gas and heat exchange phenomena that are important to plant functions.

With the expectation that a thorough understanding of air and fluid movement phenomena in lower-G will be important to effective design of advanced plant culture systems, an effort was begun to develop a test bed system in which such phenomena could be studied. One of the instruments referred to above, the PGU, which was used in plant growth experiments on the STS-3 flight and was scheduled again in slightly modified form on SL-2, was evaluated first for its potential use in supporting such experiments.

The PGU was originally designed for simple containment of a large number of small growing plants with only a minor amount of monitoring or manipulation during flight. The requirements of an engineering data acquisition effort are, on

the other hand, for containment of one to a few highly instrumented plants. The problem is thus to replace much of the growth space to contain instrumentation for monitoring plant response. Figure 5 shows the original PGU flight package. Figure 6 shows an analysis of the availability of space for components of the expected experimental systems. The space available without major modification is the cavity occupied by the six PGCs (plant growth chambers). If only one or two of the PGCs are needed for containment of experimental organisms, then the remainder of the space is available for mounting of monitoring or experiment control instrumentation. There are a number of experimental operations involving primarily aspects of biochemistry or tropistic behavior which can be accommodated in the PGU under these conditions. Except for preliminary experiments on monitoring, it is of somewhat limited use in physical testing, particularly of the sort that employs optical measurements. Because it is difficult to modify for highly instrumented experiments that would require extensive rearrangement of the interior, the needs of a much broader range of tests can be better served by a more open structure. Other deficiencies of the PGU are in the absence of temperature control below ambient and in the control over quality and quantity of light.

The PGU, either in its present or derivative forms, has one other major deficiency: it is too complex and expensive to build for the number of units which are needed for routine experiment development. A basic need of nearly all flight

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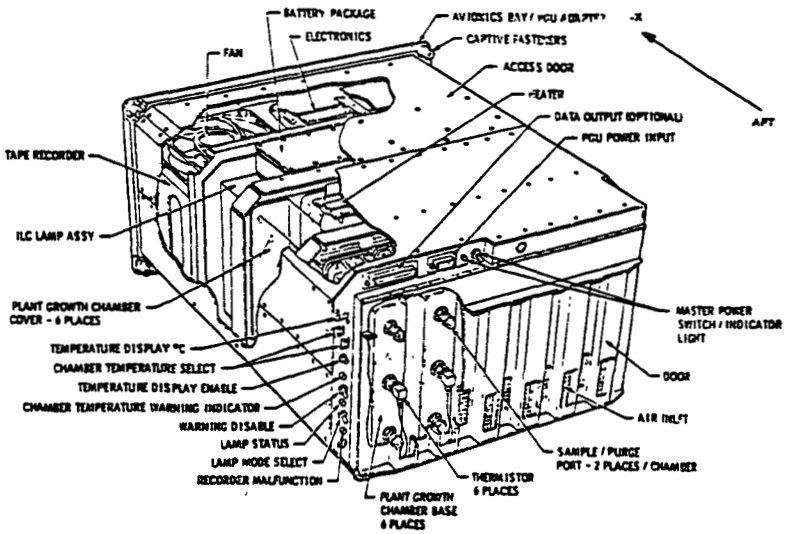

Figure 5. The Plant Growth Unit (PGU) of the STS-3 and SL-2 Lignification Experiments. Dimensions - 56 x 36 x 27 cm and sized to fit a standard middeck locker space. Weight as used on STS-3, approximately 24 Kg. Average Power as used on STS-3, 52 W at 28 Vdc. Power interface by single power cable to an outlet in the ceiling of the shuttle middeck. Source: V.S. Clifton, 1982. Spacelab Mission 2 Experiment Descriptions-Second Edition. NASA TM-82477. NASA George C. Marshall Spaceflight Center.

experimenters is for an easily obtainable, inexpensive, and well-standardized experiment container which can be used in the laboratory for experiment development and then integrated into a flight program with minimal effort.

The design shown in Figure 7 is a first attempt at meet-

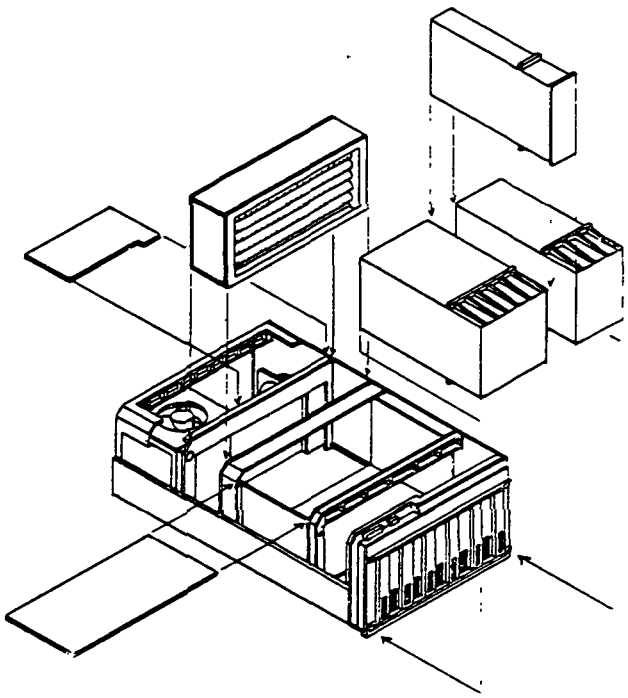

Figure 6. Blow-up analysis of space available for use in the PGU. Drawing is not to scale.

ing a set of requirements common to both our own specific line of experimentation and the general run of basic science flight experimentation with small plants. Our own experiments require a flexibly open space in which to mount a variety of test fixtures. The system used in development and exercising of the design is a system for optical monitoring of small plants, plant surrogates, gas or fluid experiments in which differences in density occur. The experimental support platform consists of a monocoque frame of aluminum sheet and honeycomb plastic built to the dimensions of a full locker tray and thus capable of being inserted into a standard locker. Side members of sheet aluminum formed into tubes serve as air channels for cooling of the interior. Inlet and exhaust screens are mounted in the modified locker door. The end caps

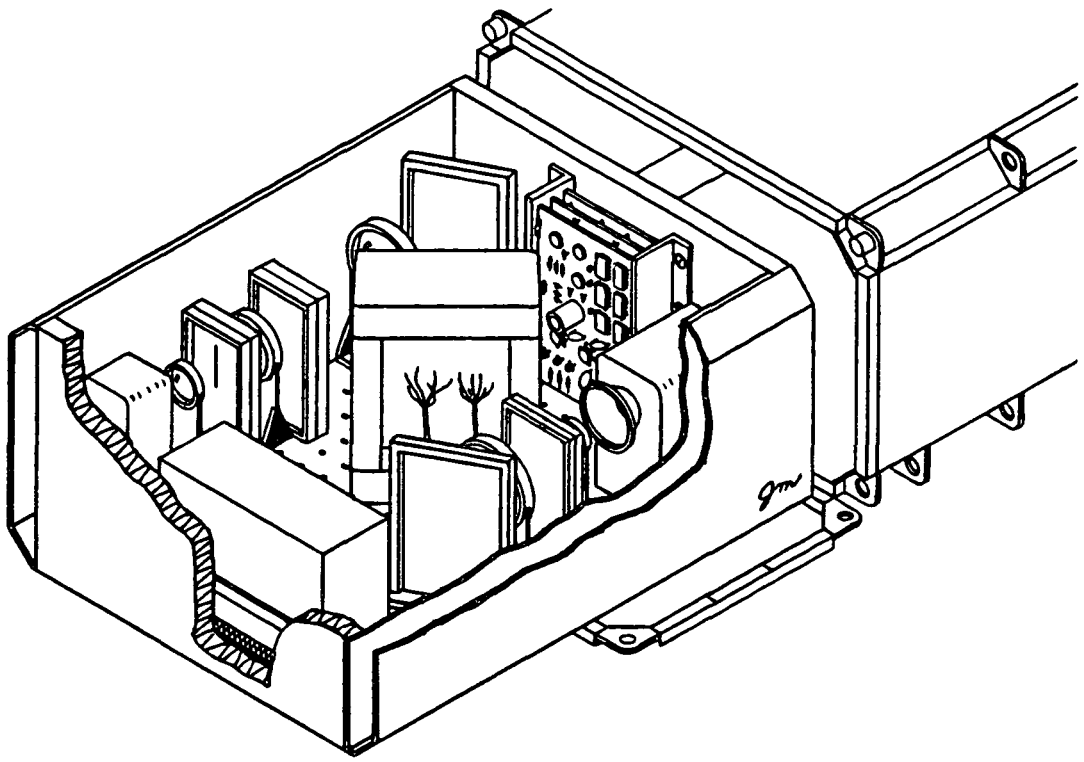

Figure 7. Plant experiment platform designed to support engineering data acquisition on the interaction of plants with the zero-g environment. The optical system is based upon a design by W. M. Poteet of System Specialists, Tucson, Arizona.

of aluminum covered lexan honeycomb provide additional rigidity. The side frame and the end caps are mounted with a regular array of channels or brackets which serve as attachment points for a variety of instruments. The version shown mounts an optical bench configured for color Schlieren optical monitoring of air movements around small plants mounted in a small wind tunnel instrumented for control of air velocity, temperature, humidity and atmospheric gas composition. The optical system was designed by W. M. Poteet of System Specialists, Tucson, Arizona. Continued development is aimed at the incorporation of microprocessor control of experiment functions and data acquisition.

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Electrochemical Control of pH in a Hydroponic Nutrient Solution

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ABSTRACT

The pH of hydroponic nutrient solution is usually controlled by addition of dilute acid or base solutions. In a CELSS, this sort of control would eventually produce an accumulation of the elements composing the acid and base. This paper describes the utilization of an electrochemical cell for pH control, and discusses its effects on hydroponically grown lettuce.

INTRODUCTION

In a functional CELSS system, a large number of environmental variables will require careful monitoring and control. One environmental variable of major importance for the higher plants will be the pH of the hydroponic nutrient solution. During the growth cycle of higher plants, individual nutrient elements are taken up in different amounts, and at varying rates. As a result, the pH of the nutrient solution changes. Additionally, plants release a variety of organic compounds into the nutrient solution, and these compounds also change the pH of the solution. Consequently, the pH of the nutrient solution shifts with the changes produced by the uptake/release of substances in the nutrient solution.

INTRODUCTION

Nutrient solution pH is usually controlled by adding either dilute acid or base to maintain the desired value. There are two problems with this technique in a CELSS, however. First, the use of acid and base for pH control adds the chemical elements of those compounds to the nutrient solution, and eventually may produce an accumulation of those elements. The effects of this accumulation have not been determined experimentally for higher plants, but in bacterial chemostats, such accumulations can retard the growth of the cultured cells (1). Secondly, the use of dilute acidic or basic solutions requires that suitable concentrates be carried along with the CELSS unit in order to mix the required solutions. These concentrates could add significantly to the amount of mass required to assemble a functional CELSS.

An alternative method of pH control, the use of an electrochemical cell, circumvents both of these problems. The use of an elctrochemical method of pH control presents a possible problem, however, in that the compounds in the nutrient solution could potentially be affected by the flow of electrons. The focus of this paper is to describe an experiment in which conventional chemical pH control was compared with electrochemical pH control, with regard to both the efficiency of the electrochemical system and to any effects it might have on the growth of higer plants.

MATERIALS AND METHODS

Seedlings were prepared for the experiment by germinating Lettuce (Lactuca sativa, cv. Grand Rapids) seeds between sheets of filter paper suspended over standard ASHS nutrient solution (2). Opaque plastic covers were placed over the seeds to maintain humidity and enhance hypocotyl elongation. The covers were removed 4 days after sowing, and 3 days later the seedlings were transfered to an NFT nutriculture system located inside a controlled environment room. Each seedling was held in the nutriculture system in a polyurethane foam plug, treated to prevent toxicity to the seedlings (3). Two identical nutriculture systems were used, each consisting of a 120 l nutrient reservoir, a magnetically coupled pump to circulate the solution, a supply manifold of PVC pipe, 4 troughs (1.5 m long by 12.7 cm wide) made of PVC vinyl gutter, and a drain manifold of PVC which returned the nutrient solution to the reservoir. The troughs for the control and experimental groups were arranged in alternating order across the width of the environment room to minimize the effects of any environmental gradients on the experiment. Trough covers were made from 3mm thick PVC vinyl sheet, cut to cover each trough completely. Each cover was drilled with 31 holes (1.6 cm diameter) on 3.8 cm centers through which the polyurethane plugs holding the seedlings were inserted. For each experiment, 100 l of modified Hoaglands solution (2) was used in each resevoir. Nutrient solution was maintained at a constant volume in both reservoirs during the experiment by a level-controling float

relay connected to a pump and a make-up resevoir filled with deionized water. Water use was monitored by recording the amounts of make-up water added.

Environment room air temperature was controlled at 25/20°C D/N. Relative humidity was maintained at 70%. CO2 concentration was monitored and controlled at 1200 ppm. PAR was supplied by four 400 W metal halide HID and two 400 W high pressure sodium lamps, producing an average total irradiance of 550 $\mu mol/m^2/s$. Photoperiod was 16h/8h D/N.

The pH control system was identical for both nutrient solutions, and consisted of a pH electrode, a pH controller with high and low limit switches (Chemtrix 45e), and the pH controlling hardware. In the control reservoir, the hardware consisted of two peristaltic pumps, one connected to a reservoir of 0.1N HCl, and the other connected to a reservoir of 0.1N KOH. In the experimental reservoir, the pH control system consisted of two platinum wires, one placed directly in the nutrient solution, and the other placed in an agar bridge partially submerged in the solution. The two wires were connected to a DC power supply (1).

In the conventional pH control system, the pH controller applied power to the acid or base pumps to add the required chemical. In the electrochemical reservoir, the pH controller added "acid" or "base" by turning on one of a pair of relays which determined the polarity of the electrodes and applied a voltage across them. The relays applied a voltage of either +45 V DC or -45 V DC across the platinum electrode wires,

depending on whether the pH was too high or too low.

Plants were harvested at 14, 21 and 28 days of age. For the first two harvests every other plant from each trough was removed, thus thinning the plants as well as providing data on growth. For the third and final harvest, all the remaining plants in each trough were removed. Fresh weights were determined, the plants dried to constant weight at 60°C, and the dry weights measured.

RESULTS

Figure 1 illustrates the record of nutrient solution pH for the two nutrient reservoirs. As can be seen from this figure, the electrochemical cell lost some control capability near the end of the experiment.

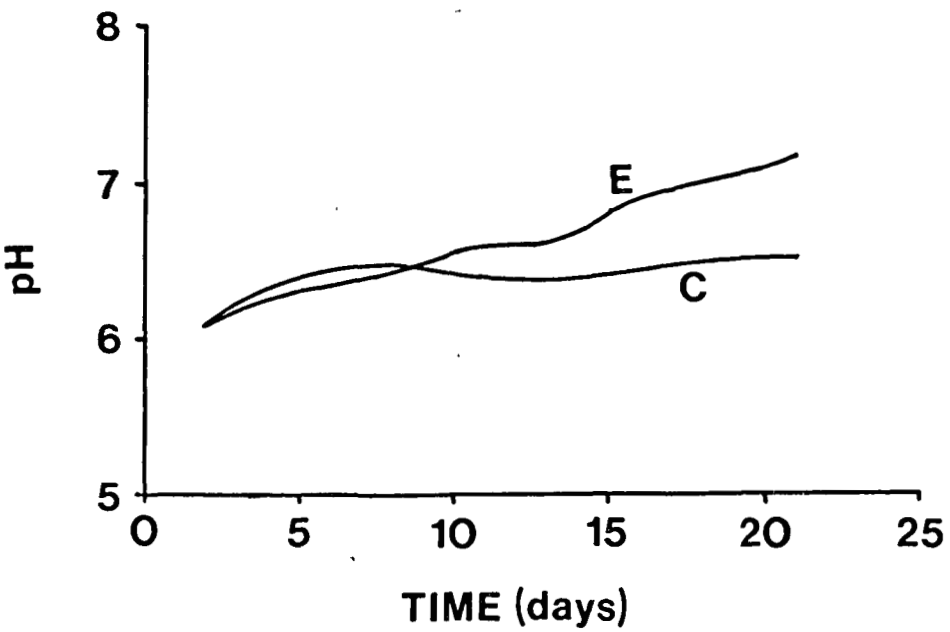

Figure 1. Nutrient solution pH versus time for control (C) and electrochemically-controlled (E) reservoirs.

Table 1 presents biomass data collected from the lettuce plants harvested from the control and experimental treatments. These data indicate that both the control and experimental treatments produced the same results with regard to plant growth.

Table 1. Average sizes of harvested plants for control and electrochemically-controlled (experimental) groups.

Harvest Age (Days)	Fresh Weight(gm)		Dry Weight(gm)	
	Control	Experimental	Control	Experimental
14	0.43	0.40	0.03	0.03
21	9.21	9.44	0.57	0.60
28	74.29	71.85	3.49	3.37

DISCUSSION

The most serious problems encountered with the electrochemical pH control method seem to be related to the design of the agar bridge. This bridge design worked well at low current densities, but near the end of the experiment did not operate at sufficiently high current flow to correct the pH, and consequently the nutrient solution pH tended to drift out of bounds. Additionally, the limited volume of electrolyte in the cell (approximately 200 ml) required replacement every 24 hours in order to maintain a sufficient pool of electrolytes for pH control.

Despite these difficulties, the electrochemical control method worked well, and there were no detectable differences between the lettuce plants grown in the electrochemical system and the conventional control system. Apparently, there was no accumulation of toxic or inhibitory compounds in the electrochemically controlled nutrient solution. This finding provides support for the idea of using electrochemical pH control for CELSS applications.

From the pH record in Figure 1, however, it seems advisable to develop a new electrode design for long term use. This development is currently in progress.

CONCLUSION

The electrochemical pH control system described here was found to provide a feasible alternative method of controlling nutrient solution pH for CELSS applications. The plants grown in nutrient solution in which the pH was controlled electrochemically showed no adverse effects. Further research into the design of a larger capacity electrode bridge for better control is indicated by the results of this experiment, and is currently under way.

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AN ENGINEERING ANALYSIS OF A CLOSED CYCLE PLANT GROWTH MODULE

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ABSTRACT

An engineering computer model has been developed at Battelle to simulate the performance of a controlled environment agriculture system. This computer model, called SOLGEM, was developed to provide a dynamic performance simulation of an enclosed growing system in which the ventilating and air conditioning power required to maintain ideal growing conditions was provided entirely by solar energy. The model is made up of 11 primary subroutines which simulate the performance of the major components of the system, including a plant growth and evapotranspiration model, ventilation and flow model, evaporative cooling and solar-driven absorption cooling, and photovoltaic power generation.

The SOLGEM model has been applied to calculate the performance of a plant growth module similar to the module under study at NASA Ames. The plant growth module is a ground-

based plant growth research facility, intended for the study of bioregenerative life support theories.

This report presents the results of a performance analysis of the plant growth module. The estimated energy requirements of the module components and the total energy are given. The water balance and estimated plant evaporation are presented. An analysis of the effect of design alternatives of component sizing and energy use is discussed.

INTRODUCTION

The SOLGEM model is a numerical engineering model which solves the flow and energy balance equations for the air flowing through a growing environment, assuming quasi-steady state conditions within the system. SOLGEM provides a dynamic simulation of the controlled environment system in that the temperature and flow conditions of the growing environment are estimated on an hourly basis in response to the weather data and the plant growth parameters. The flow energy balance considers the incident solar flux; incoming air temperature, humidity, and flow rate; heat exchange with the roof and floor; and heat and moisture exchange with the plants.

A plant transpiration subroutine has been developed

to simulate the heat and moisture transfer between the plants and the air. This routine provides a realistic model of transpiration and leaf temperature in terms of incident solar flux, air temperature, air-flow velocity, roof and floor temperature, and humidity. Important plant characteristics such as stomatal opening, convective and radiative heat exchange, and canopy development are all included in the model.

The purpose of the SOLGEM dynamic simulation model is to provide an engineering estimate of the performance of a controlled-environment growing system. The required input to the model is a complete physical description of the system, i.e., size of all components, performance characteristics of the energy subsystems, and the weather data. For a given system design and a specific location, the hourly air temperature, humidity, and solar intensity experienced by the plants are determined. The results indicate whether or not the growing enviromnent maintained by the system was satisfactory for optimum plant growth.

The simulation model also determines the quantities of mass and energy flowing between components. The sizing of components can be accomplished by monitoring the component output and noting excesses or deficiencies. In most cases, the performance of the overall system will be affected by varying

the size until the desired system performance is achieved.

The model can also be used to conduct parameter sensitivity studies. By varying the value of a specific input and repeating the simulation run with all other parameters fixed, it is possible to determine the sensitivity of the system performance to changes in this parameter. Sensitivity studies can be used to determine the most important design variables, and to indicate the benefits possible by making changes in system design.

APPROACH TO ANALYSIS OF PLANT GROWTH MODULE

Plant Growth Module

The SOLGEM model has been used to conduct an engineering analysis of a simplified version of a plant growth module. A sketch of the module is presented in Figure 1. The module simulated consisted of a growing space 1m wide, 1m tall, and 5m long. A bank of grow lights provided radiation. The air was circulated by a single fan which passed air over a cooling coil before cycling it back to the growing space. The cooling coil removed the thermal energy added by the grow lights, and condensed and removed the moisture transpired by the plants.

Modeling Plant Heat Transfer

In an enclosed growing environment the plants are a major factor in determining the energy balance of the system. The plants absorb the incident radiant energy and convert it to thermal energy, and to latent heat in the form of water vapor. (A small percentage of the energy is converted to chemical energy by the plants. This energy is negligibly small and consequently has not been included in the analysis.)

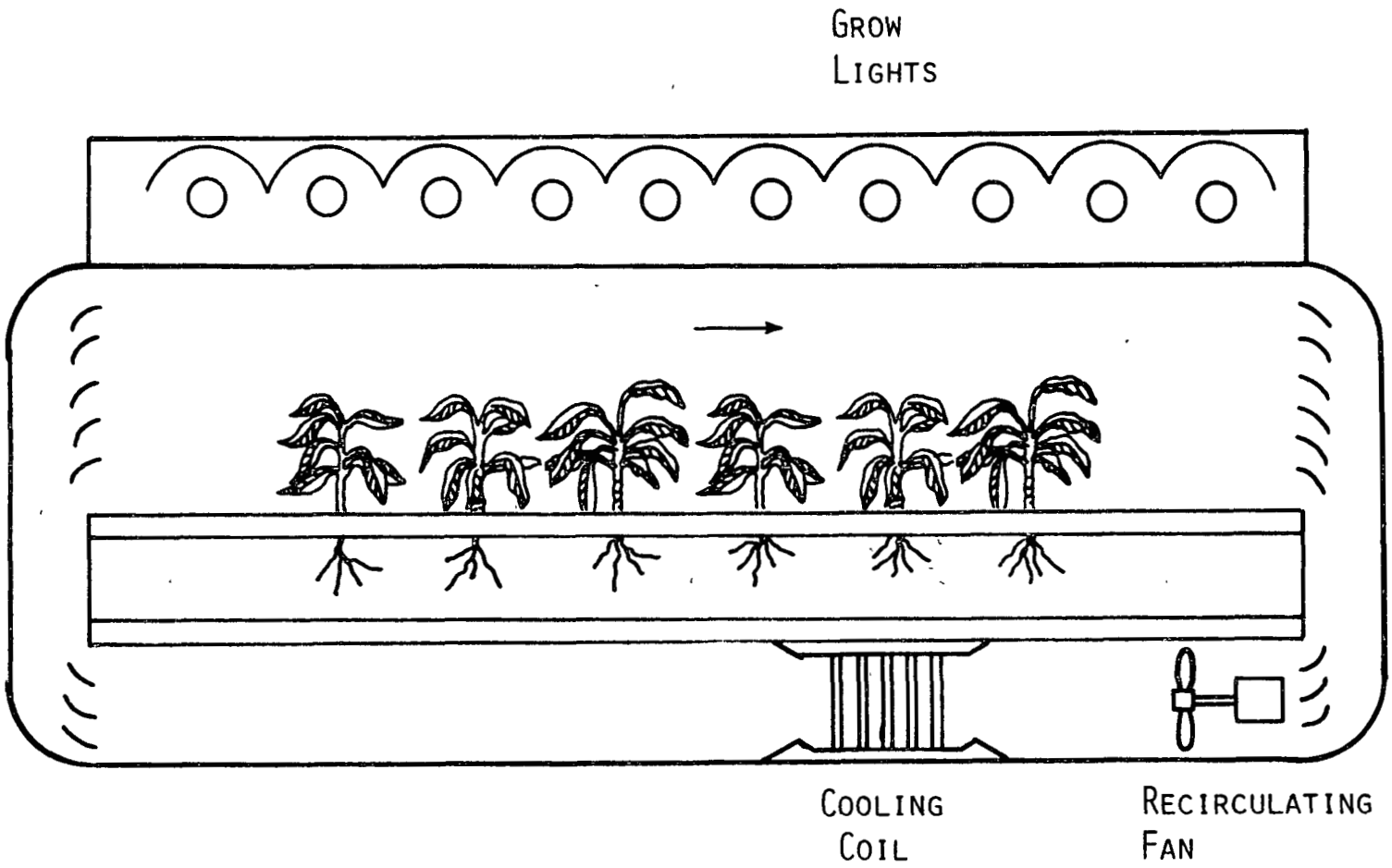

FIGURE 1. SKETCH OF PLANT GROWTH MODULE

The transpiration and leaf temperature model of Gates[1,2] was used to calculate the energy balance of the plants. The Gates model considers three energy transfer mechanisms:

- o Convection
- o Radiation
- o Transpiration

The convection of energy between the leaf and the air stream is governed by the temperature difference between the leaf and the air, and by the air flow velocity. The convection coefficients depend on the leaf geometry and are given by Gates.

The radiative exchange between the leaf and its surrounding is determined by considering five radiation sources as shown in Figure 2. The primary radiant energy input is QSOL, which in this case is the radiation supplied by the grow lights. Radiative exchange with the roof and the floor is included, as is the reflected radiation from the floor. Radiation from surrounding leaves is also considered in the energy balance.

Transpiration by the leaves is actually a mass transfer mechanism and is a significant term in the plant energy balance. The amount of energy that is absorbed by transpiration can range from 1/3 to 1/2 of the incoming radiation. The Gates transpiration model depends on leaf temperature,

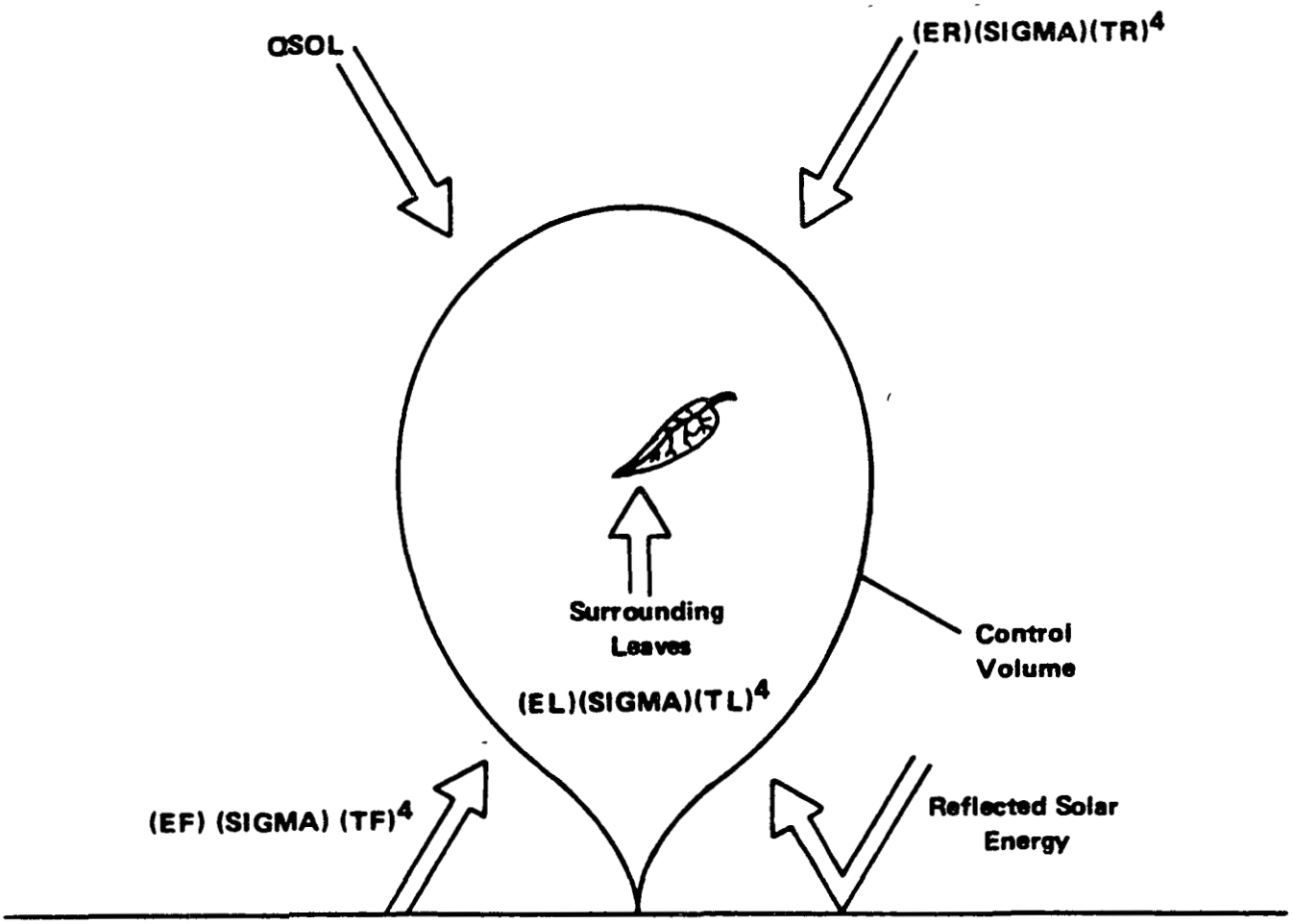

FIGURE 2. RADIATIVE HEAT TRANSFER TERMS
INCLUDED IN THE LEAF ENERGY BALANCE

air temperature and humidity, air flow velocity, and a leaf stomatal resistance term.

The stomatal resistance is an attempt to simulate the opening and closing of the plant stomate, shown in Figure 3. The stomatal resistance was selected so the predicted water transpiration rate of a mature plant canopy matched the known performance of a greenhouse. The nighttime stomatal resistance of 500 sec/cm was recommended which virtually eliminated transpiration at night. The daytime value of 5 sec/cm was found to result in a transpiration rate sufficient to absorb 1/2 of the incoming solar radiation by a mature plant canopy during full sun conditions.

Description of Component Models

Cooling Coil. The cooling coil consists of a liquid-to-air heat exchanger using chilled water as the liquid cooling medium. The amount of air passing through the heat exchanger was varied by bypassing a portion of the recirculation air around the heat exchanger. The two air streams were then mixed together before reentering the growing zone.

This simple system is capable of providing dehumidification and removal of thermal energy in one process. By varying the chilled water temperature and the fraction of the recirculation air that is bypassed, it is possible to achieve

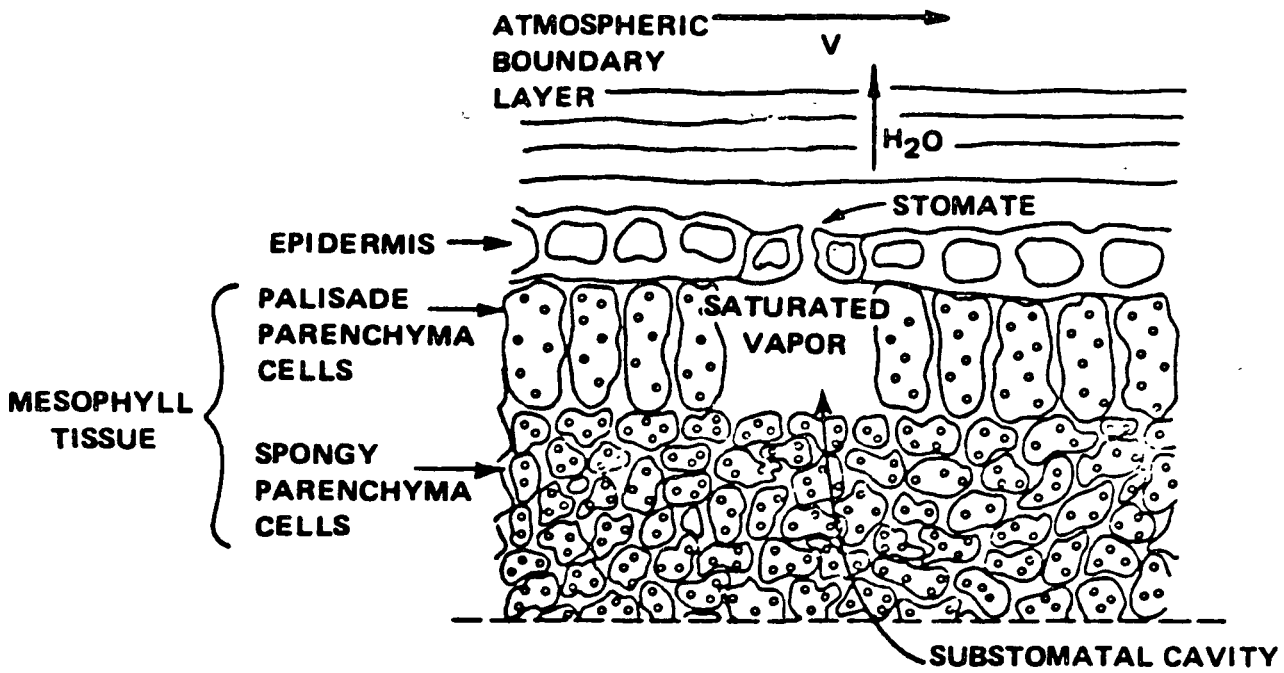

FIGURE 3. LEAF CROSS SECTION

desirable levels of temperature and humidity in the growing zone for all realistic conditions of radiation input and plant transpiration. A detailed discussion of this process follows in a later section.

Grow Lights. The grow lights provide radiant energy input to the plant canopy. In addition to triggering the growth process, which is not included in the SOLGEM model, the radiant energy affects the leaf temperature and the amount of water vapor transpired by the plants.

For the purposes of this simulation, the grow lights were assumed to be 80% efficient, meaning that 80% of the energy input to the grow lights was emitted as radiation, and 20% was given off as thermal energy. The radiant energy was transmitted to the plants, then to the air by convection and transpiration. The thermal energy was transmitted directly to the air by convection from the lights.

Circulation Fan. The electric energy supplied to a fan motor is transfered to the air stream in three distinct forms: kinetic, potential, and thermal. Kinetic energy is imparted to the air by the fan blades creating a moving air stream. Potential energy is imparted to the air by the fan blades in the form of the pressure rise across the fan. Thermal energy is imparted to the air stream in several

ways. Viscous frictional heating of the air stream occurs because of the interactions between the air and the fan surfaces. Conduction to the air stream of the thermal energy generated by mechanical friction and electrical resistance also occurs.

For a particular air flow rate, the kinetic energy is proportional to the velocity squared. The air velocity through the fan can be reduced significantly by using larger diameter fans. The required flow rate and pressure rise can be achieved by selecting the appropriate fan speed. Thus by using large-diameter, variable-speed fans the kinetic energy can be minimized.

Potential energy considerations lead to the need to minimize the product of flow rate and pressure rise. The flow rate requirements are fixed by the thermal energy input of the grow lights. However, the pressure rise is a function of the system design. Thus a low pressure rise system is desirable.

The temperature rise of the air through the fan system results from frictional effects and inefficient hardware. Thus fans and motors having the highest efficiency should be selected for the plant growth module.

Given a particular plant growth module design(i.e., the pressure drop is a function of the air flow rate), the fan energy must be minimized by minimizing the air flow requirements, and by choosing the appropriate number and size of fans.

Temperature and Humidity Control Strategy

The air passing through the growing zone absorbs the heat generated by the grow lights and increases in temperature. The air also absorbs the moisture transpired by the plants. All the thermal energy and moisture absorbed by the air must be removed as the air passes through the recirculation ducts.

Air Flow Velocity. The air flow velocity can be increased by increasing the recirculation fan speed. As the air flow velocity increases the temperature rise across the growing zone decreases. Thus the air flow velocity can be used to control the allowable temperature variation across the growing zone.

Chiller Control. Two chiller control variables are required in order to condition the recirculation air. By varying the chilled water temperature and the fraction of air bypassing the chiller, the temperature and humidity

of the recirculation air can be controlled. Lowering the chilled water temperature and increasing the bypass increases the amount of water removed relative to the amount of sensible heat. Raising the chilled water temperature while reducing the bypass will result in more sensible heat reduction and less water removed.

RESULTS OF ANALYSIS

An analysis of the plant growth module was conducted for an illumination level of 1000 μmol/sec/m^2. The temperature of the air exiting the growth zone was fixed between 25 C and 26 C. The relative humidity was held between 60 and 70 percent.

The air temperature rise through the 5 m plant growth module is shown in Figure 4. For the mature plants, the temperature rise is less than 1 C at an air flow rate of 60 m^3/min. This is equivalent to 1 m/sec flow velocity.

For seedlings the amount of water transpired is significantly less. Thus the temperature rise at a given air flow rate is greater than for mature plants.

Figure 5 presents the average leaf temperature as a function of air flow rate. The average leaf temperature appears to be slightly less sensitive to air flow rate than the air temperature. Thus increasing the air flow velocity is not as beneficial to reducing plant temperatures as would be indicated by calculating air temperature only.

The effect of cooling water temperature on the humidity

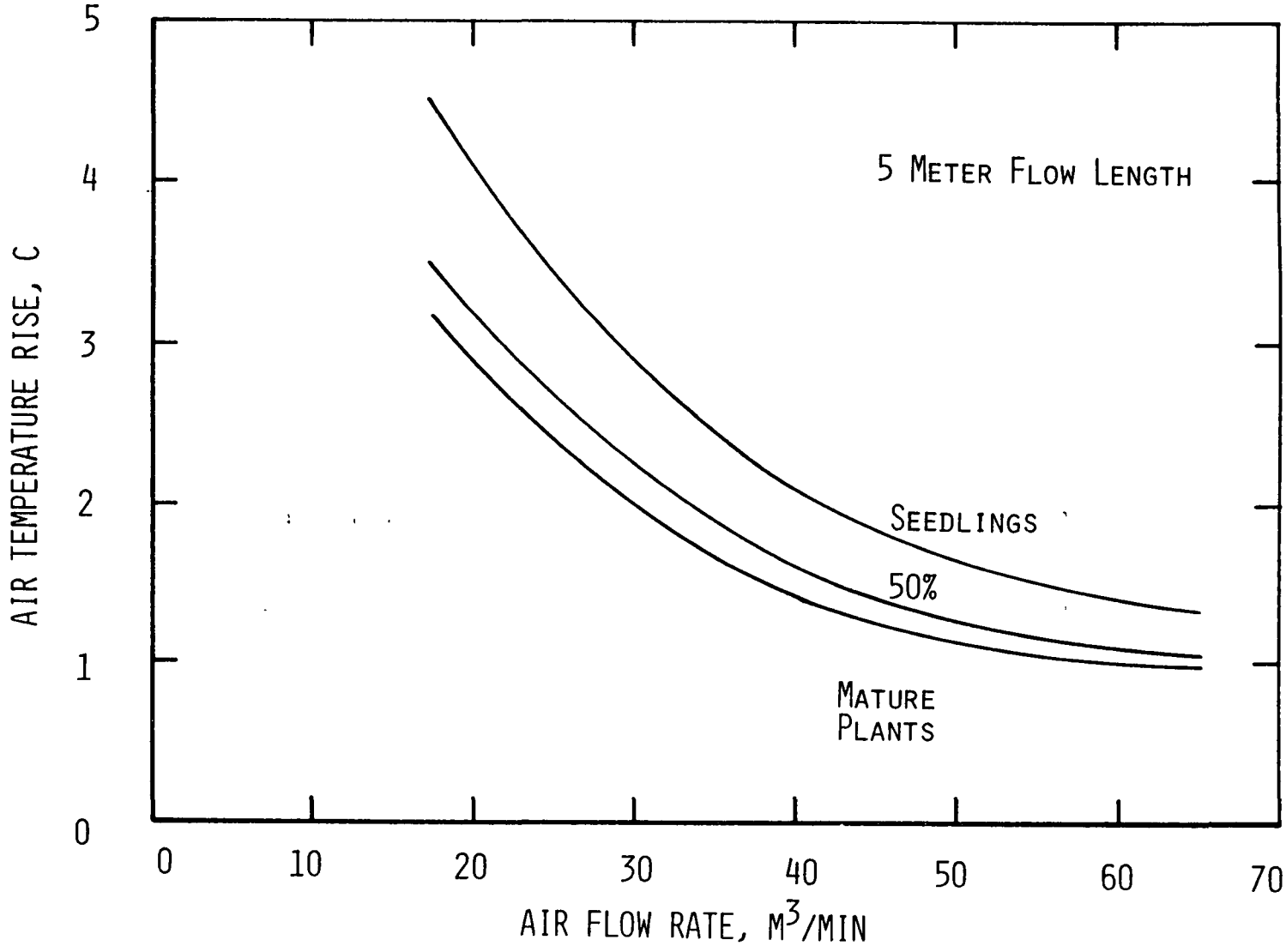

FIGURE 4. AIR TEMPERATURE RISE THROUGH PLANT GROWTH MODULE

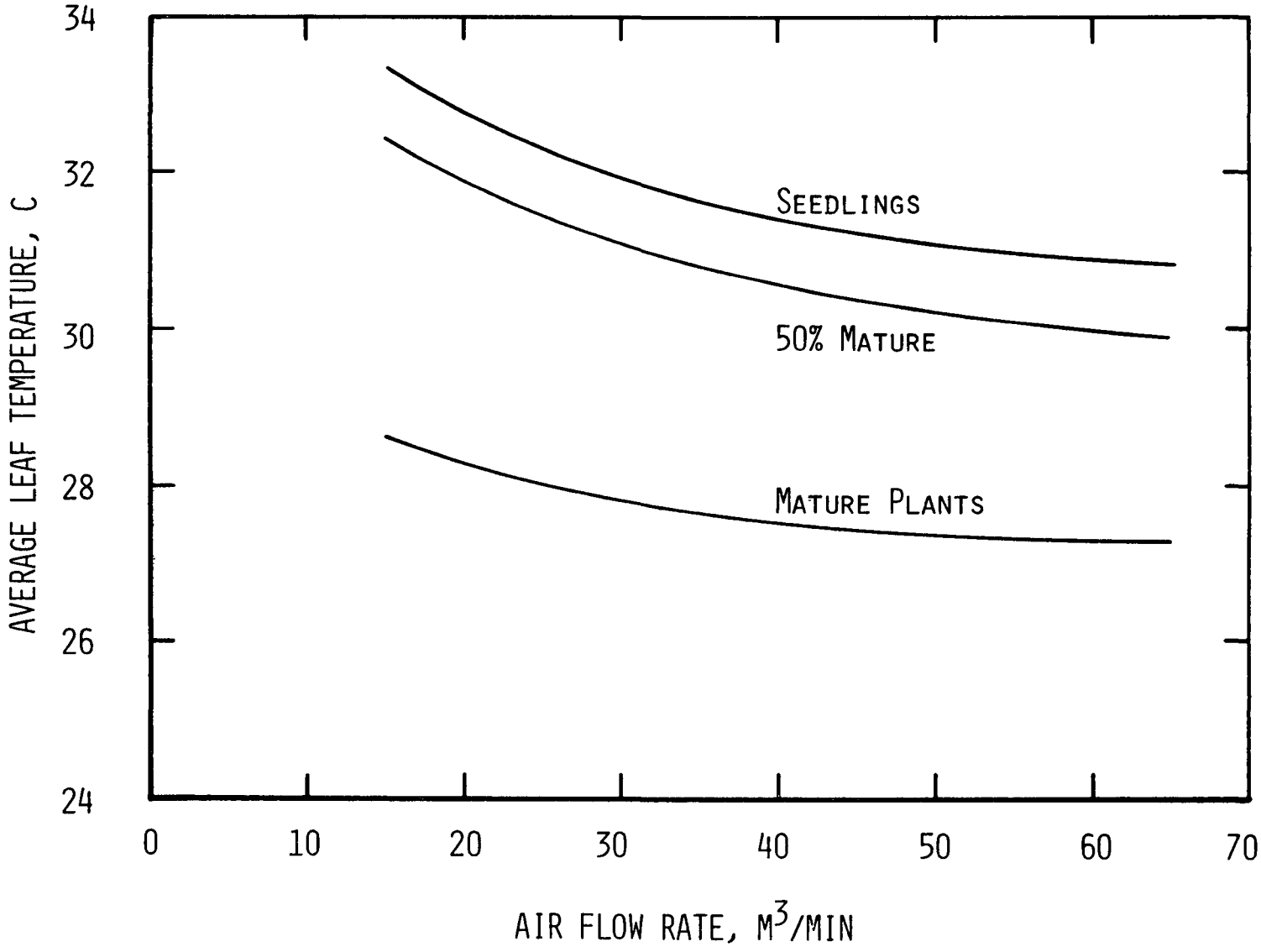

FIGURE 5. AVERAGE LEAF TEMPERATURE

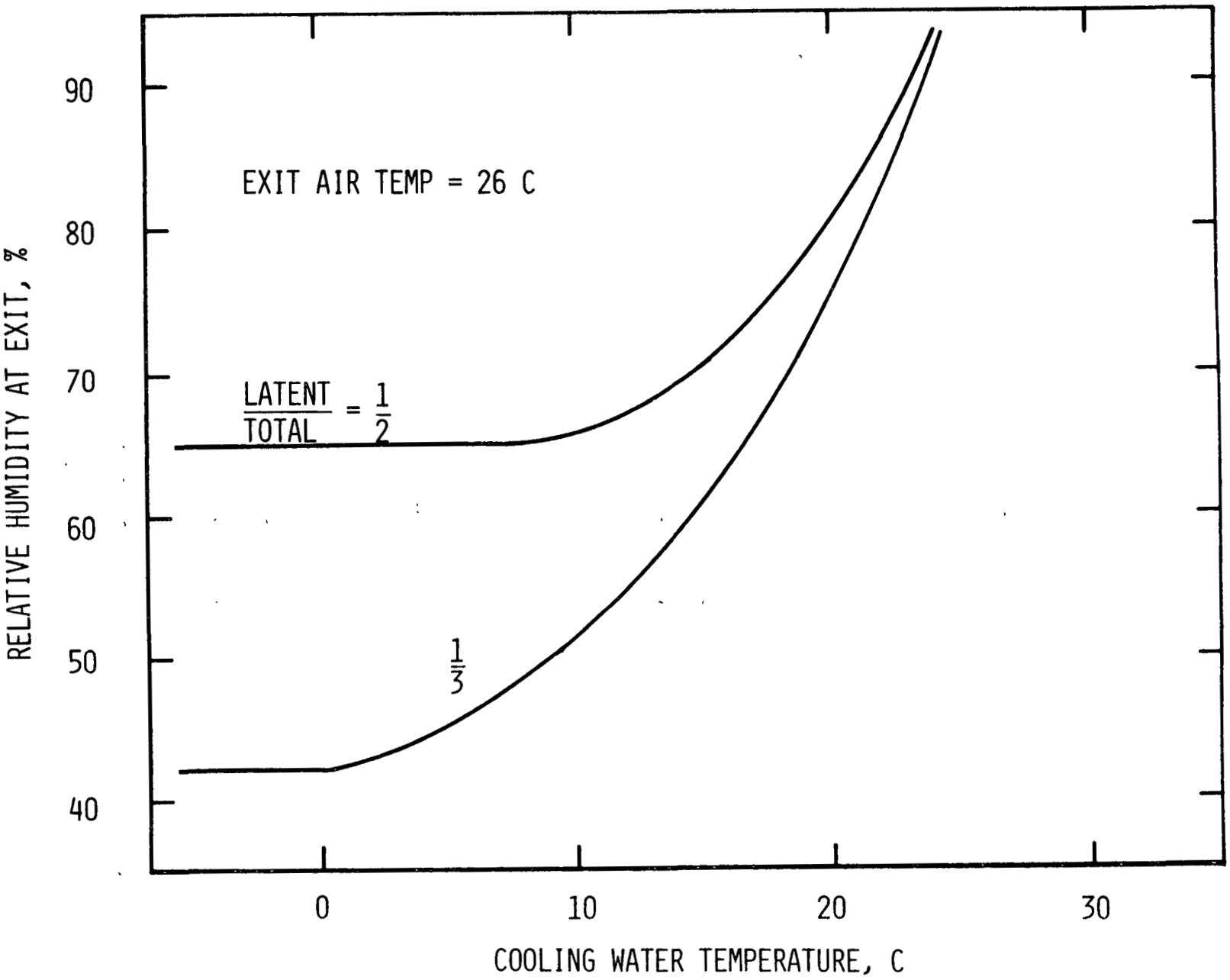

FIGURE 6. HUMIDITY LEVEL VERSUS COOLING WATER TEMPERATURE AND LATENT HEAT RATIO

level in the growing zone is presented in Figures 6 and 7. For practical levels of the latent heat ratio(the ratio of energy absorbed by transpiration to the total energy absorbed by the air stream), it is possible to achieve humidities down to 65 percent with a cooling coil. At lower transpiration rates characteristic of young plants, the humidity could be controlled with water near room temperature.

Figure 7 shows the effect of air temperature on cooling water temperature. For higher air temperatures in the growing zone, the allowable cooling water temperature is higher. Higher cooling water temperatures result in lower energy requirements for humidity control.

Energy Requirements. The energy requirements for the plant growth module are given in Table 1. Shown are the estimates for a 5 m and a 30 m module. Lighting is the major energy user, although the energy required for cooling is significant. The fan energy is relatively small for the 5 m module, but becomes sizable when scaled up to 30 m. The 30 m module is assumed to have the same cross section area in the air return ducts as the 5 m module and the same air flow velocity. The fan energy could be reduced by 2 or 3 by enlarging the air return ducts and cooling coil.

Water Usage. The total water transpired by the plant

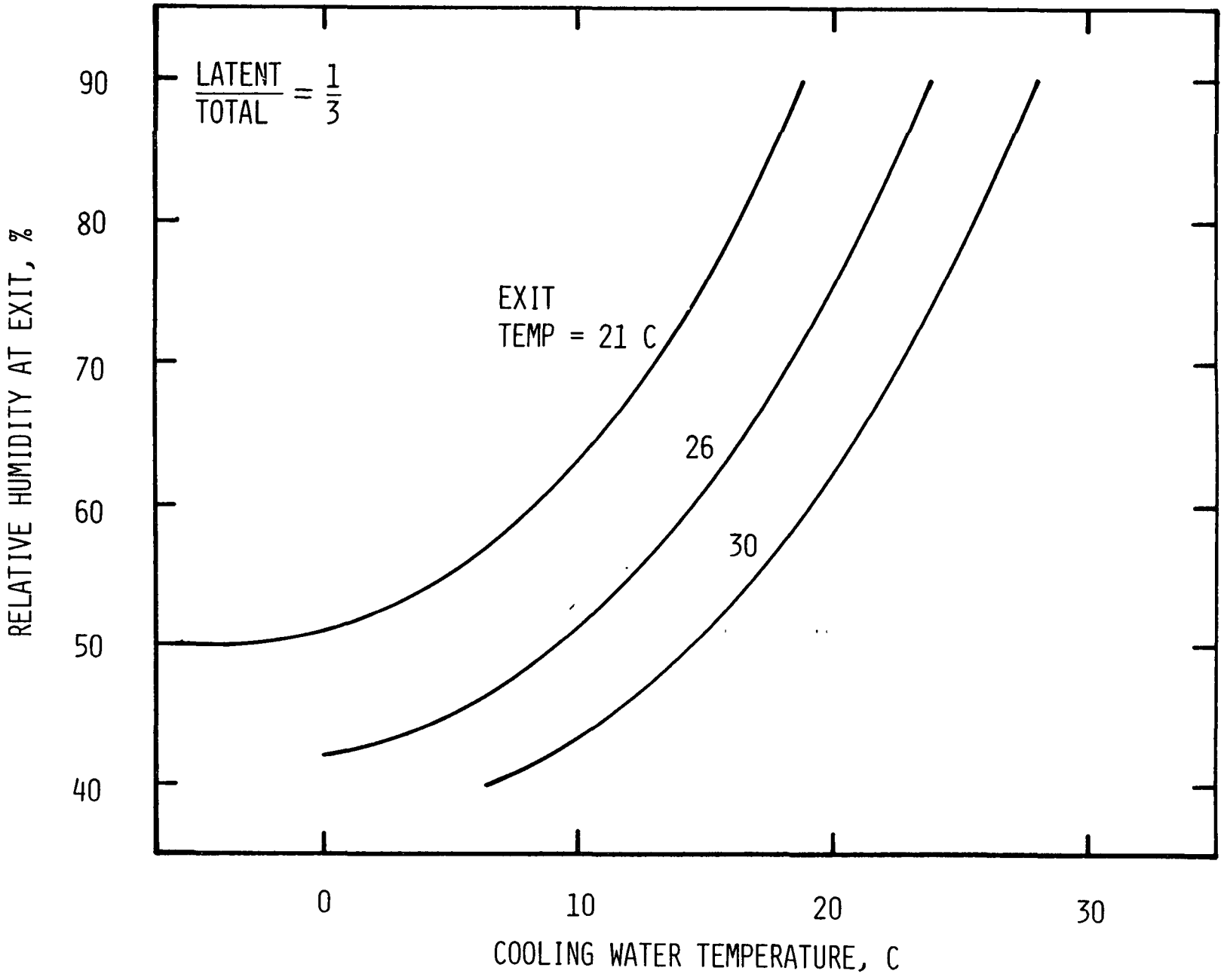

FIGURE 7. HUMIDITY LEVEL VERSUS COOLING WATER TEMPERATURE AND AIR TEMPERATURE

Table 1. Plant Growth Module Energy Requirements.

	Total Power,kw	Lighting %	Cooling %	Fan %
5 meter	2.5	69	27	4
30 meter	16.0	65	25	10

growth module filled with mature plants is estimated to be approximately 850 gm/hr. This level of transpiration, coupled with the light energy input, results in a latent heat ratio of 1/3.

CONCLUSIONS

The temperature and humidity of a closed-cycle plant growth module can be controlled with a circulating fan and a chilled water cooling coil. The fan speed controls the temperature rise of the air passing through the growing zone. The amount of thermal energy removed from the recirculating air can be controlled by the fraction of air passing through the cooling coil, the remainder being bypassed. The temperature of the circulating water controls the amount of moisture removed, thus controlling the humidity level in the growth zone.

Minimizing the energy requirements of the plant growth module will require minimizing the air flow rate, and reducing the pressure drop through the recirculation ducts. It will also require operating the cooling coil in the most energy efficient mode. If the chilled water is generated by a heat pump, the higher the chilled water temperature, the more efficiently the heat pump will operate. Thus it will be most efficient to operate the plant growth module at the highest acceptable temperature and humidity.

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WHOLE SYSTEM RECYCLING AND CONTROL

Dr. Robert D. MacElroy, Chairman

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Gas and Water Recycling System for IOC Vivarium Experiments

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Abstract

The animal and plant experiments for the Japanese life science mission at the initial operational capability of space station and the associated gas and water recycling units for supporting these experiments were investigated. Based on these investigations, preliminary design was conducted. The interfaces between animal experiment facility, large scale phytotron and these units and the scale of these units such as estimated power requirements, capacity and so on are described.

1. Japanese life science missions

Many life science experiments are proposed by Japanese researchers to be conducted in the Japanese experiment module (JEM) which will be attached to the space station common module. These experiments are classified into three time phased missions such as the IOC phase, mid phase and growth phase. In the IOC phase 28 experiments (1),(2) are proposed so far and they are also classified

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into four fields such as biology, space medicine, CELSS and biotechnology. The gas and water recycling units are used to support biology, CELSS and medicine experiments which use small animals, plants and algae in the IOC phase. Table 1 shows the missions proposed in Japan. Main experiment items using small animals are as follows.

- Mechanism of space motion sickness and its countermeasure.

- Musculoskeletal degradation mechanism and its countermeasure.

- Change of kidney function and its countermeasure.

- Blood and immunological change and its countermeasure.

Table 1. Proposed Missions

	Species	Research Field
Small Animal	Rat, Mouse, Squirrel Monkey, etc.	Medicine/Biology
Higher Plant	Lettuce, Spinach, Soy bean, Potato, Tomato, etc.	Biology/CELSS
Algae	Spirulina Chlorella	CELSS

Main experiment items using plants and algae are as follows.

- Development, maturity, flowering, fructification and geotropism of plants.

- Morphological and metabolic change of algae and plants necessary for controlled ecological life support system (CELSS).

- Optimum cultivation technology in space for CELSS.

2. System Requirements

According to the concept study of Japanese experiment module so far, a single rack space will be provided for the experiments using small animals and plants respectively and a half rack space for the experiments of algae. This module concept design will perhaps be changed in the phase B study, but seems not to be changed drastically. Therefore, the study for the required ability of water and gas recycling units depends on the concept design of Japanese module. In a single rack six squarrel monkeys can be bred and 45cm x 60cm cultivation area can be used for plants and also for a half rack space 2 litter volume cultivation can be used for algae[3],[4].

Estimated amounts of metabolism of these animals, plants

C-3

Table 2. Metabolism of Proposed Missions

(unit: Kg)

			Rat (10)	Squarrel Monkey (6)	Plant ($45x60cm^2$)	Algae (2 lit.)	Total
Intake Quantity	Water	1 day	0.3	0.48	0.2	0.1	1.08
		90 days	27.0	43.2	18.0	9.0	97.2
	O_2 Gas	1 day	0.09	0.15	-	-	0.24
		90 days	8.1	13.5	-	-	21.6
	CO_2 gas	1 day	-	-	-	0.1	0.1
		90 days	-	-	0.6-1.0	9.0	9.6-10.0
Exhaust Quantity	Feces	1 day	0.14		-	-	
		90 days	12.6		-	-	
	Urine/ Waste Water	1 day	0.15	0.25	-	-	0.4
		90 days	13.5	22.5	30.0-50.0	2.0-4.0	68.0-90.0
	Trans-piration	1 day	0.15	0.23	0.2	0.1	0.68
		90 days	13.5	20.7	18.0	9.0	61.2
	CO_2	1 day	0.1	0.18	-	-	0.28
		90 days	9.0	16.2	-	-	25.2
	O_2	1 day	-	-	-	0.07	0.07
		90 days	-	-	0.4-0.7	6.3	6.7-7.0

and algae are shown in Table 2.

3. Composition and function of the system

The system consists of two main processing units, one of which is the gas recycling system and the other is the water recycling system. The block diagram is shown in Fig. 1. Only the interface between these units is a water pipe line, through which condensed water from the dehumidifier of the gas recycling unit is fed to the storage tank in the water recycling unit. Therefore, these units can be operated separately.

(1) Gas recycling system[3]

The required function of the gas recycling system is to take off CO_2 gas from the outlet air of the animal vivarium and also take off O_2 gas from the outlet air of the phytotron and the algae cultivator.
The separated CO_2 gas is stored in the tank to supply for controlling air circumstances in the phytotron and the algae cultivator. The separated O_2 gas is also stored in another tank for supplying to the animal vivarium.
The function diagram is shown in Fig. 2. As CO_2 gas in the outlet air from the animal vivarium is gradually increasing, a part of the outlet air is directly returned to the animal vivarium and the residual air is led to the

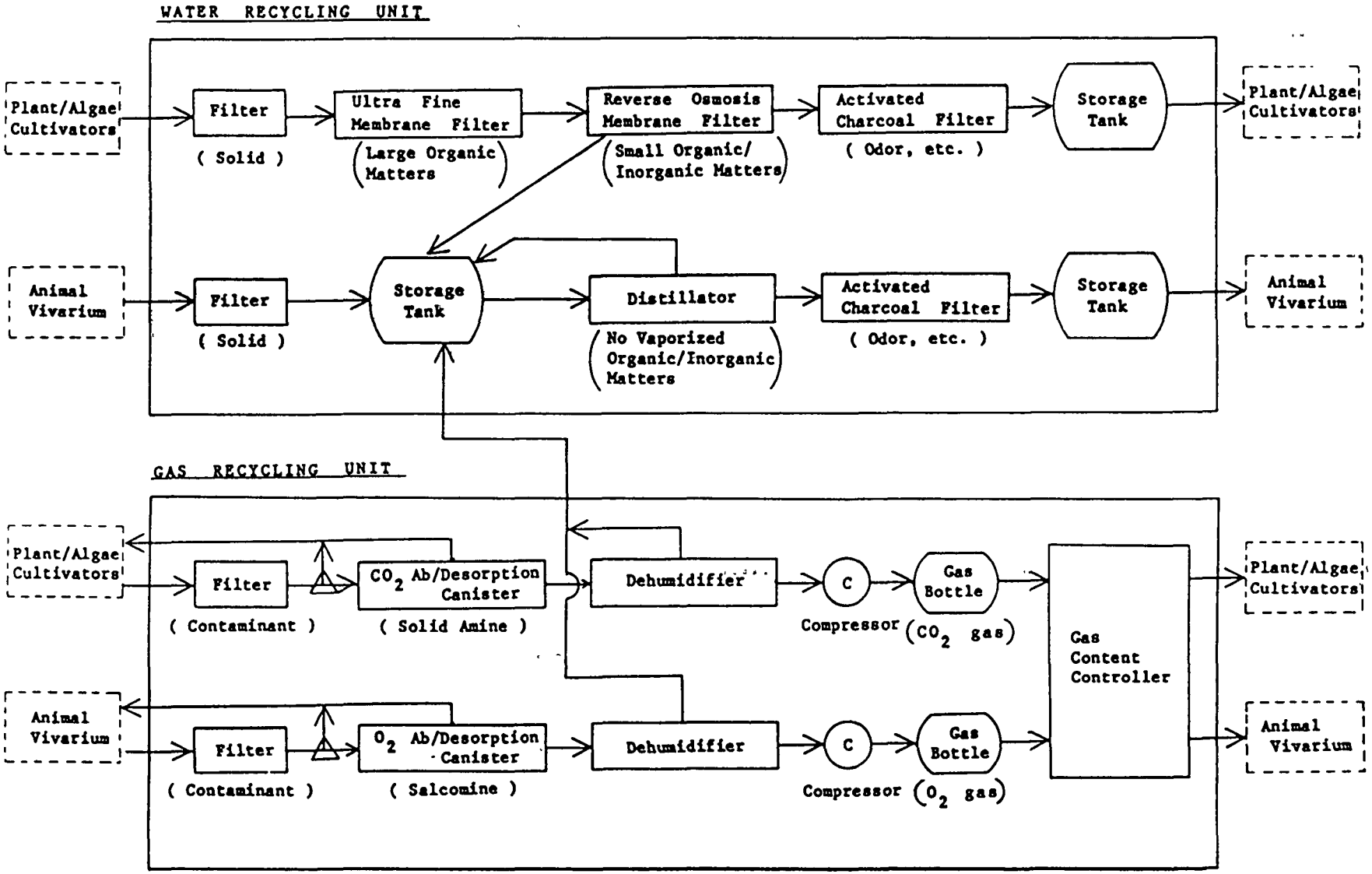

Figure 1 Functional Diagram of Gas and Water Recycling System

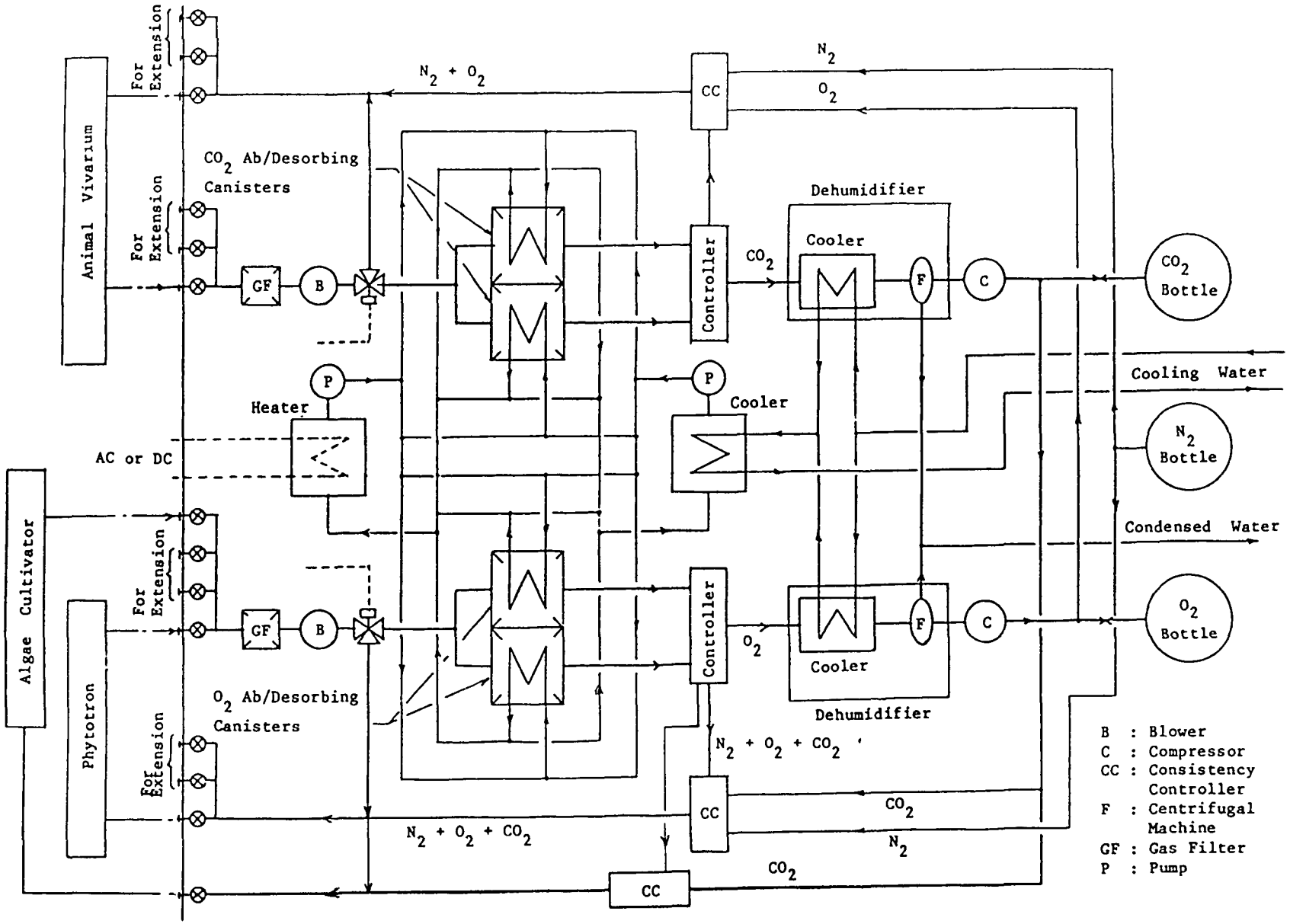

Figure 2 Block Diagram of Gas Recycling Unit

canister filled with solid amine which has the characteristics to absorb and desorb CO_2 gas according to the change of temperature. One of the typical characteristics of solid amine is shown in Fig. 3. The dividing rate of the outlet air flow is controlled by the flow controller according to the required CO_2 gas content which is planning to set below 0.5%.

To operate the process continuously, two canisters filled with solid amine are provided. When one of them works in CO_2 absorbing phase, the other works in CO_2 desorbing phase. Switching is automatically controlled by the event program.

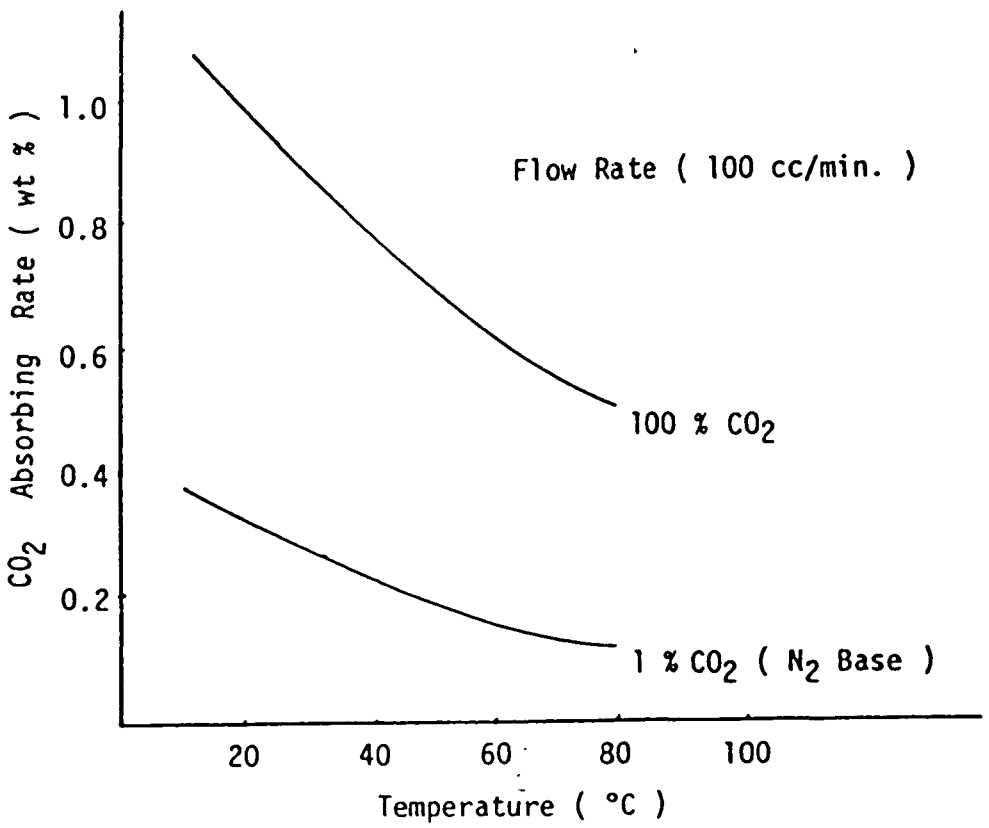

Figure 3 Solid Amine CO_2 Absorb/Desorbing Characteristics

As O_2 gas in the outlet air from the phytotron and the algae cultivator is also slowly increasing, a part of the outlet air flow from those experiment facilities is directly returned to the phytotron and the algae cultivator, and the residual air is led to the canister filled with salcomine which has the characteristics to absorb and desorb O_2 gas according to the change of temperature.

One of typical characteristics is shown in Fig. 4. The dividing rate of the outlet air flow is controlled by the flow controller according to the required O_2 gas content which is not determined now.

To operate the process continuously, two canisters filled with salcomine are provided. They work as same as the CO_2 gas processing system.

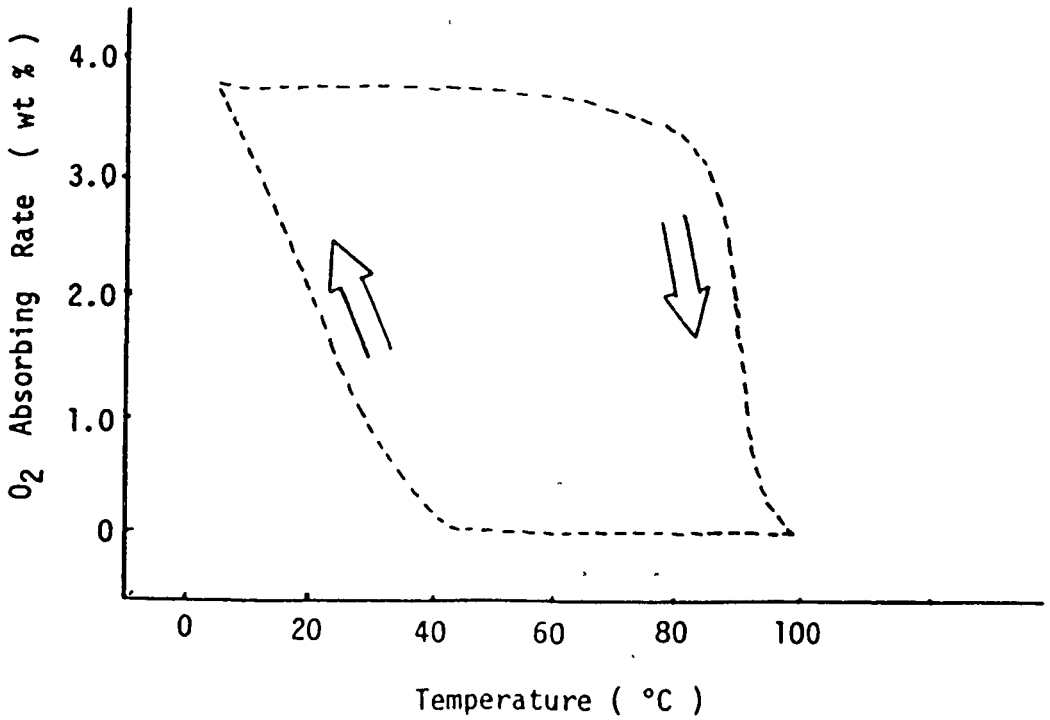

Figure 4 Salcomine O_2 Absorb/Desorbing Characteristics

To use these two kinds of canisters continuously during at least three months, some improvements on the characteristics of solid amine and salcomine are supposed to be necessary. This gas recycling system does not include the control system for temperature and humidity of the supplying air to the experiment facilities. In each experiment facility, suitable air conditioning is independently demanded for providing the different air condition in each phase of the experiments. On the other hand, to control the gas contents in each facility separately makes the system and operation very complex and is causative of miss operation. Therefore, the concentrated method for gas contents control to each experiment facility seems to be better than the separated individual control method.

(2) Water recycling system[3]

The required function of the water recycling system used in the IOC phase is to process urine from the animal vivarium and wasted nutrient solution from the phytotron and the algae cultivator and also to make the clean water enough to use as drinking water for animals and as the water to make refreshment of solution for plants and algae.

The waste liquid from the experiment facilities contains many kinds of organic matters, inorganic matters and

little solids such as pieces of roots, therefore, those materials must be removed. The functional diagram of the water recycling system is shown in Fig. 5.

As the drinking water for animals requires high purification level comparing to the water to make nutrient solution for plants and algae, the system consisted of two processing lines shown in Fig. 5 is chosen in order to make process effectively. Only the condensed liquid at the reverse osmosis membrance filter unit is fed to the distillator unit in order to recovery water much more.

The role of the ultra fine membrance filter is to remove large proteins. The reverse osmosis membrane filter is used to take off small molecular weight organic and inorganic matters such as NaCl and so on which pass through the ultra fine membrane filter. The purified water passed through the reverse osmoisis membrane filter is stored and used for plant and algae experiments. The residual condensed liquid is fed to the storage tank and mixed in the liquid coming from the animal vivarium. The mixed liquid is fed to the distillator to take off consistencies which are not vaporized. The residual organic matters and odor contained in the purified water from the distillator are finally taken off by the activated charcoal.

The residual liquid at the distillator is sent to the crystalizer and taken off crystalloid consistencies and fed back to the storage tank again.

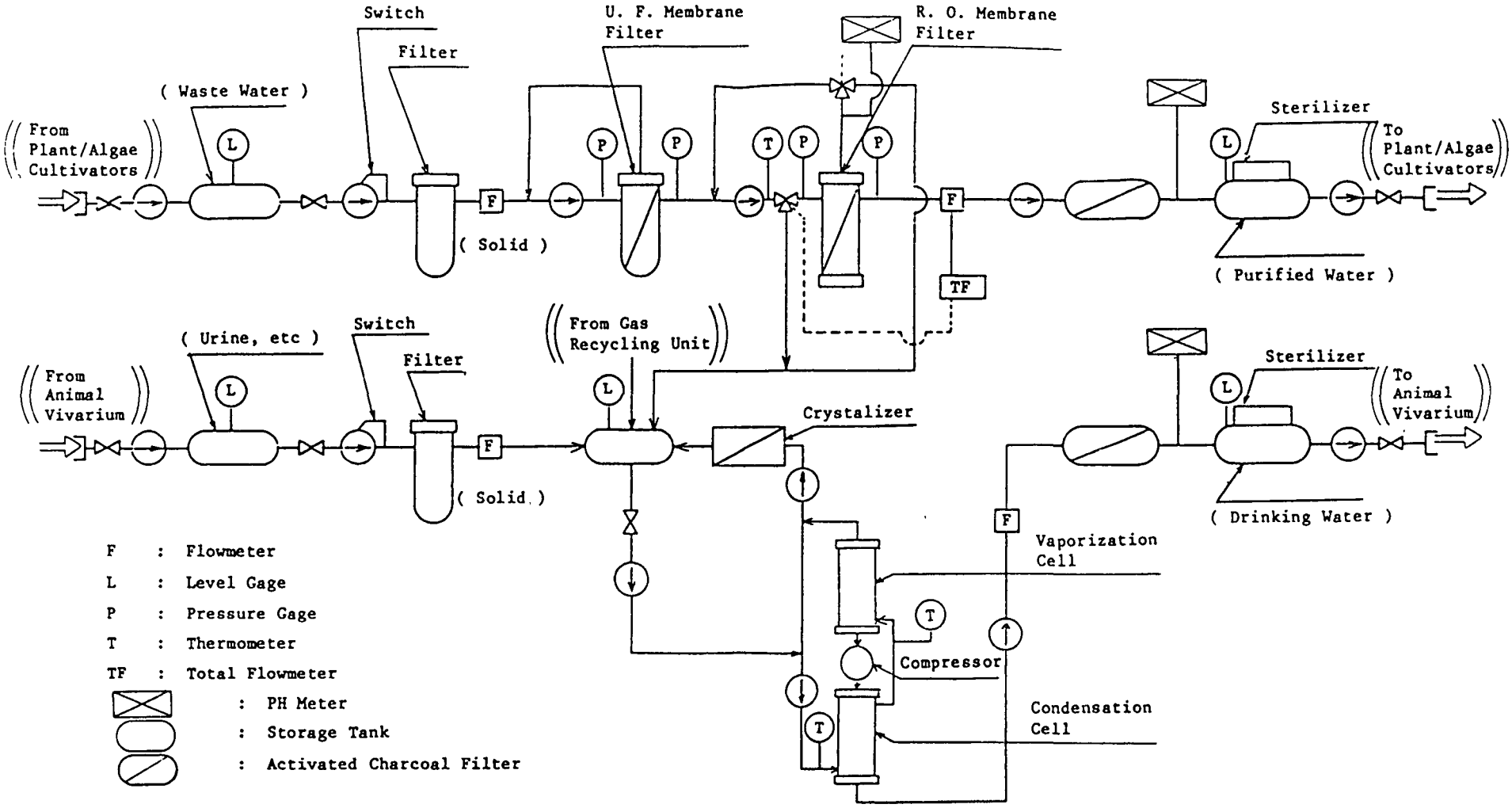

Figure 5 Block Diagram of Water Recycling Unit

The estimated volume of waste water from the plant and algae experiment facilities is about 26 litters and it is fed to the water recycling system every 15 days and processed in about six hours. On the one hand, as the estimated volume of liquid from the animal vivarium is about one litter every day, it is stored in the storage tank and processed every five days.

4. Specifications of the system

The specifications of the water and gas recycling units are shown in Table 3 and the configuration is shown in Fig. 6.

In order to use as a common experiment support facility of the life science missions, as shown in Fig. 7 it is

Table 3. Specification of Each Unit

			Water Recycle Unit	Gas Recycle Unit
Volume			1/2 Single Rack	1/2 Single Rack
Weight (Kg)			250	200
Processing Capacity			5 lit./every 5 days 26 lit./every 15 days	CO_2:0.33 Kg/day O_2: 0.22 Kg/day
Power Requirement	Monthly Average	(Kw/day)	4.22	12.13
		(Kw/hr)	0.18	0.51
	Monthly Total (Kwh)		127	364
	Peak (Kw)		0.52	0.56

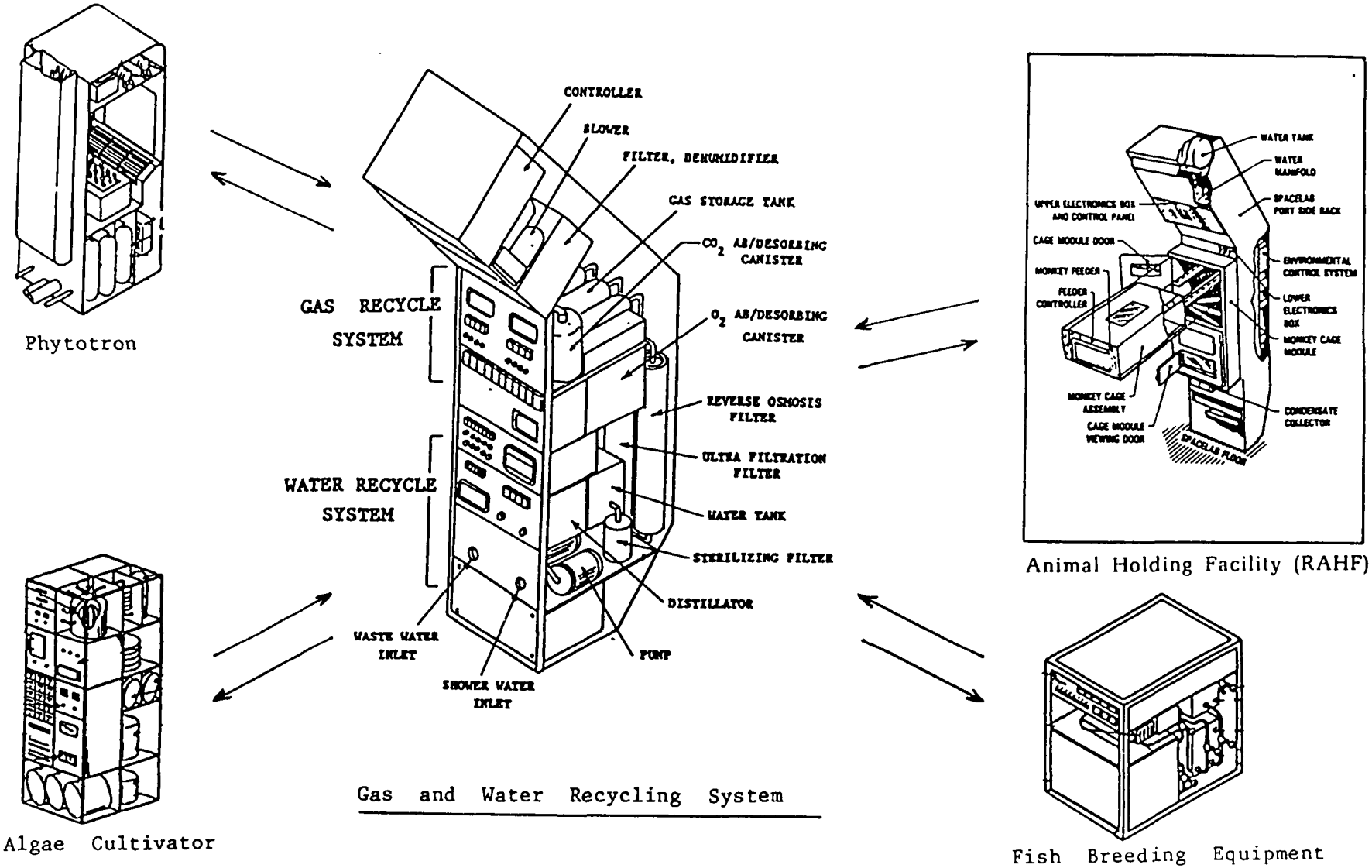

Figure 7 Gas and Water Recycling System and Related Life Science Experiment Equipments

designed that the interfaces to the experiment facilities are only pipes and signal lines.

To make easily exchange the degraded canisters and filters, the cassette styles are adopted. As the minimum exchange interval, three months are planned.

5. Conclusion

These water and gas recycling units is designed as one of the common experiment support system for the life science experiment facilities used in the Japanese Experiment Module, and to use these units will save transportation and operation costs for the life science experiments in the space station.

These units are also designed to have so simple interfaces that the connection to another life science experiment facilities such as the Research Animal Holding Facility (RAHF)[5] developed by the Rockheed Missiles & Space Company shown in Fig. 7 can be easily done with small modifications.

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WATER RECYCLING SYSTEM USING THERMOPERVAPORATION METHOD

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ABSTRACT

A water recycling system concept for the crew of the space station is presented. A thermopervaporation method is a new key technology used for the distillation process, utilizing a hydrophobic membrane. An experimental study of thermopervaporation revealed that the permeation depends on the gap between the membrane and the cooling surface in the condensation room: the steam diffusion occurs with gaps less than 5 mm while natural convection becomes dominant with gaps more than 5 mm . A brief discussion of the system operation is also described.

1. INTRODUCTION

The manned space station will need much water for the crew activities and experiments such as those concerning life science. The cost for transportation of clean water and waste water including urine is very expensive. For example, one crew will use about 11 tons of water a year (30 ℓ a day), which will cost about 90 million dollars just for the transportation of clean water from the ground to the space station. The introduction of the water recycling system for purification of urine and waste water will reduce the operation costs of the space station and experiments conducted in it, accordingly.

The Earth's natural recycling system is based on the natural balance of the biosphere circulation system. The biosphere activity depends on the utilization of solar energy for the circulation of air and water. In this circulation, water plays several important roles: material reservoir and transportation in state of solution or mixture; temperature control by evaporation and condensation; environmental constituents in a moisture or a liquid state. Water takes part in every material process on earth.

The enormous industrial progress and the population explosion since entering this century have revealed that the balance of the biosphere circulation system is sensitive and delicate against

external stimulants: the industrial products exhaust large amount of waste material as by-products with extremely unnatural constituents, destroying the material balance in the local ground area; the environment has, also, been destroyed by the introduction of roads in fields and mountains, by the use of agricultural chemicals and chemical fertilizer in farming, and by indiscriminate deforestation, causing an increase in desert areas. Water has also been involved in the expansion of such environmental disruption.

The movement to recover a healthy global environment has accelerated the development and manufacturing of recycling equipment/systems as well as progress in environmental science: ecological support systems have become addressed for use in the "earth" space station! Some technologies accumulated through development of recycling equipment/systems can be utilized for controlling the life support system in the space station.

The water recycling system for use in the space station can be composed of filtering processes for mid-class purification of water and a vaporation process for high purification of potable water. This corresponds to natural water recycling: the underground water or well water is purified by filtering through sands and soils; water from rivers originates in rainwater which is distilled water evaporated from the earth surface. Water used in cities, however, is mainly artificially filtrated and treated with disinfectant.

In applying the water recycling system to the space station there are many technical difficulties that arise principally from the micro-gravity condition and, the construction of a complete recycling system. The system must have the capability of preventing the mixture of gases, which degrades the system performance.

The water recycling system for the space station must be built by means of artificial technology because biotechnology still contains unknown factors, such as, hazardous effects caused under microgravity, and is lower in controllability and versatility. Moreover, limited space and limited power supplied in the space station requires small sized and low power dissipating hardware.

Other candidate systems for the space station use are summarized by Herrala, et al.[1], and in Japan's CELSS study, a conceptual study on the water recycling system has been reported[2] [3]. In our report, a candidate system configuration of the water recycling for crew usage is presented. The system presented here consists of filtration and evaporation processes, with membranes used for both types. Experimental studies on the new key technology of membrane evaporation, " thermopervaporation ", using a hydrophobic membrane are introduced.

The thermopervaporation method is , essentially, favorable to use in the space station because it includes no moving parts in the basic process.

2. STUDY ON WATER RECYCLING SYSTEMS

The amount of waters treated by a one-man crew in the space station is estimated in Table 1. The most recent membrane separation performance is characterized in Fig.1.

Table 1 Water Treatment per One-Man Crew

Intake: H_2O	3,050 g/day	(*)
Excreted: Urine	1,553 g/day	(*)
Excreted: Other	1,497 g/day	(*)
Shower	10 ~ 20 ℓ a shower	

(*) Metabolism estimated by Jackson

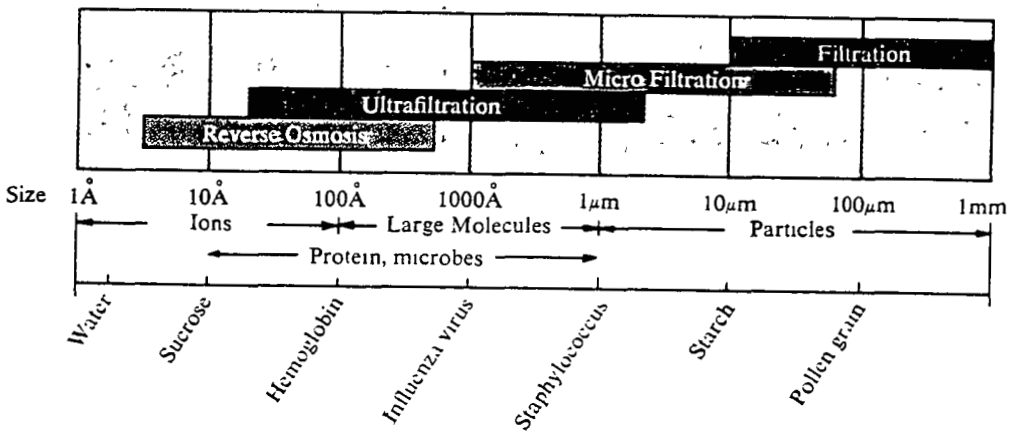


Fig. 1 Useful Ranges of Various Separation Processes

Fig.2 shows one candidate water recycling system, treating shower waste, urine, and other drainage to get shower water and potable water. First, the prefilter (micro filtration), rejects miscellaneous particles contained in the shower waste and the urine. Next , the ultrafiltration membrane rejects organic macromolecules and suspended solids, and the various solvent ions are then rejected in the reverse osmosis process. The product water from the reverse osmosis membrane is further purified by removing a trace of organic materials by means of activated chacoal, then is stored in the ultraviolet sterilization reservoir to be supplied as shower water. A portion of the permeate from the reverse osmosis membrane module is further purified by the thermopervaporation process to supply for potable water. Since the drain from the shower room includes surfactants, the feed for the thermopervaporation process is taken from the product water from the reverse osmosis membrane to prevent the membrane from losing its hydrophobic characteristic.

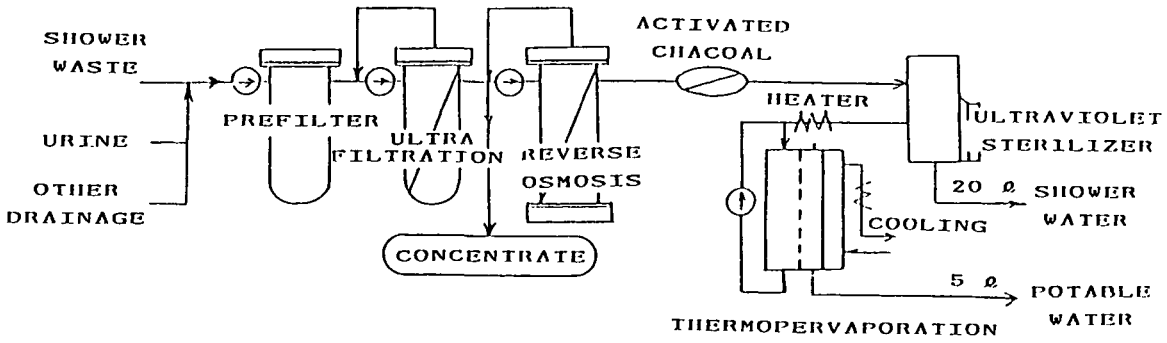


Fig. 2 Water Recycling System

This and candidate systems are shown in Table 2, and are compared and evaluated in Table 3 (system A is the one in Fig.2). The water recovery of system A is not high because the concentrate from the reverse osmosis membrane is exhausted and cannot be applied again in the recycling process. System B consists mainly of the thermopervaporation method with a crystallizer for separating solids, which results in the highest recovery, although it has the disadvantage of using more dissipation power than the other systems. Since the thermopervaporation method can use heat directly as evaporation energy, heat exhausted from various equipment in the space station can be used as the heat source and a highly reliable system is expected owing to the smaller number of components. System C is a modified configuration of system A, which includs the crystallizer to separate solids from the concentrate, and therefore realizes the highest recovery of water. The selection of system A or C depends mainly on permissible power consumption. System A is preferable from the viewpoint of the low power consumption while system C is with regard to complete water recovery. One possible treatment of the concentrate from the membrane processing is to be treated as part of the waste management system.

Table 2. Case Study of Water Recycling System

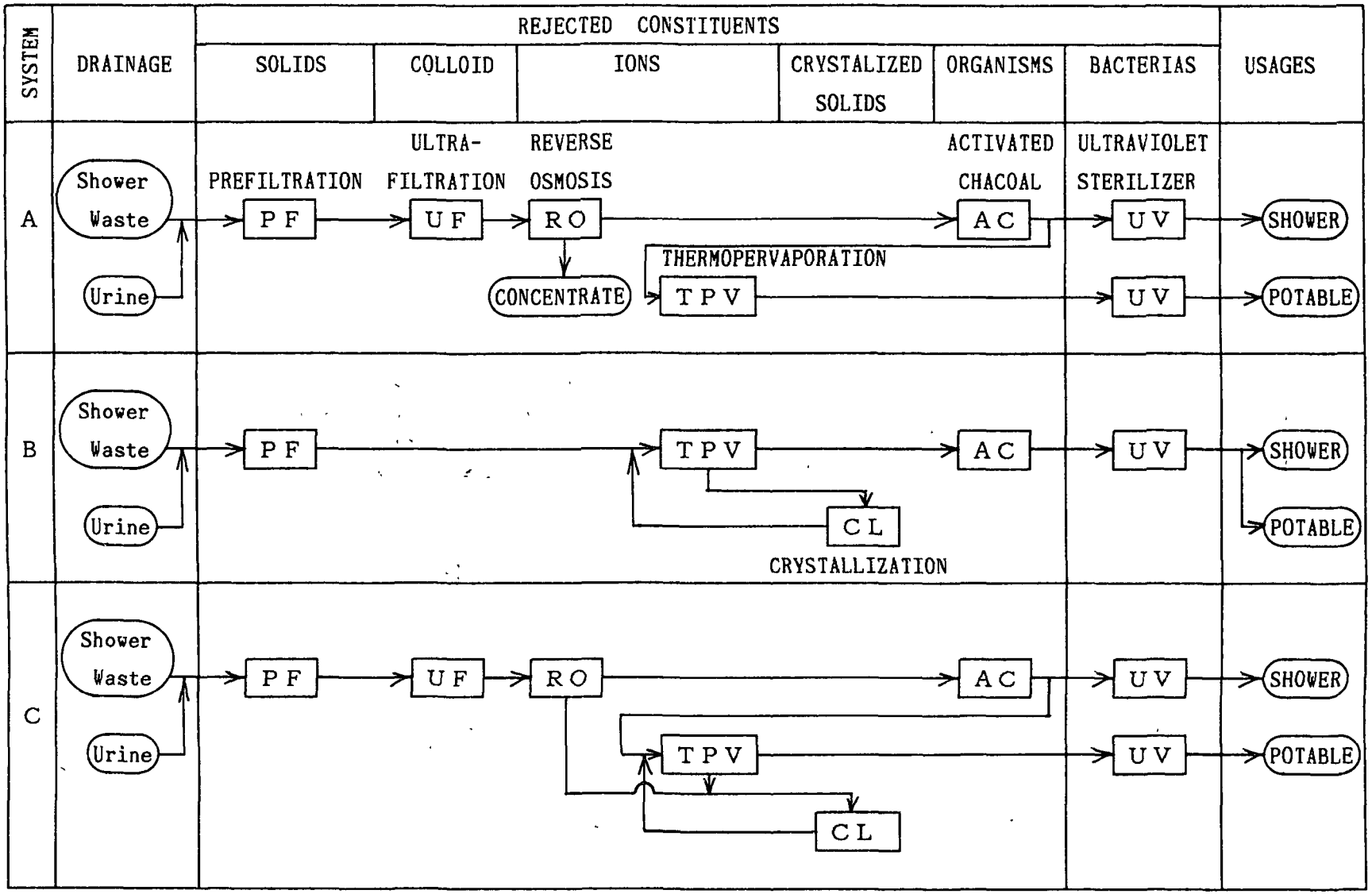


Table 3 System Comparison and Evaluation

ITEMS	SYSTEM A	SYSTEM B	SYSTEM C
WATER RECOVERY RATE (%)	80	100	100
PUMP POWER RATIO	40	1	40
TPV POWER CONSUMPTION RATIO	1	5	1
CL POWER CONSUMPTION RATIO	0	5	1
COMPONENTS NUMBER	7	5	8
FEATURES	·Low recovery ·Low power	·Small scale ·Large power	·Large scale ·Medium power

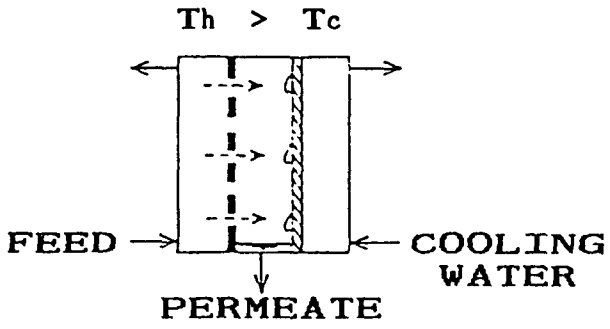
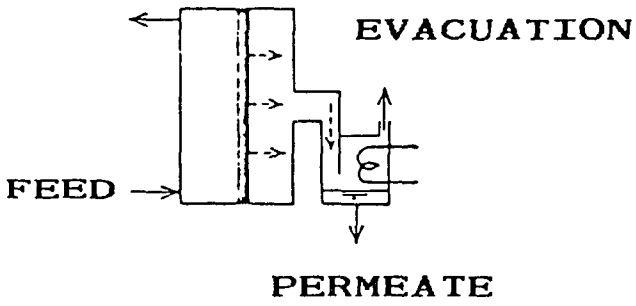
3. THERMOPERVAPORATION TECHNOLOGY

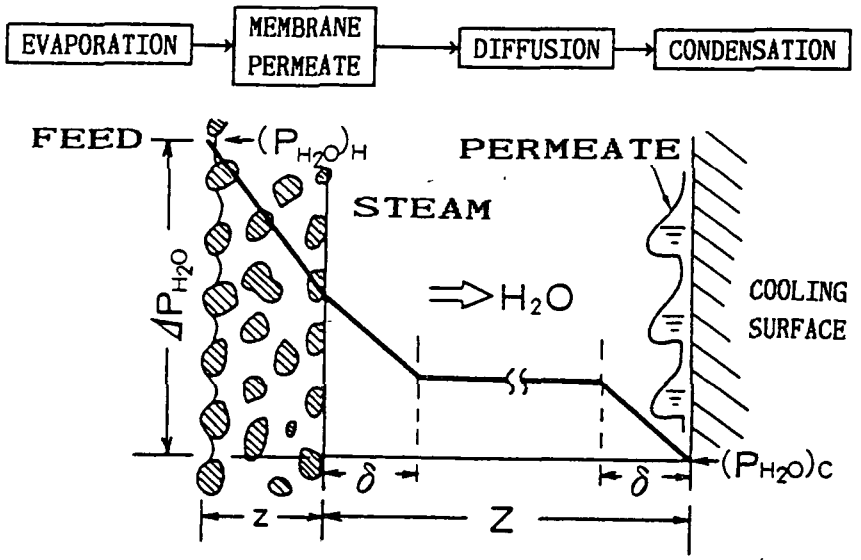
3.1 Principle and Features

The thermopervaporation method, a specialized pervaporation, is for membrane separation accompanied with phase transition. It has, however, several different features from usual pervaporation, since the porous hydrophobic membrane is used as the separation membrane. That is, the membrane has highly permeable water flux owing to its large porosity so that the ideal separation speed at a practical level is obtainable with a small temperature differential. In other words, since low-grade energy such as exhausted heat can be used as the energy source, an economical system is realizable despite the increase in consumed energy accompanied by the phase transition. Furthermore, by the multiplicative effect of vaporization and membrane separation, the separation ratio of solvents is expected to be extremely high, over 99.99 %. Since the membrane is fabricated, in general, from polytetrafluoroethylene (PTFE), it possessses excellent resistence to heat, chemicals and pH, compared with previous reverse osmosis membranes.

Table 4 shows the features of the thermopervaporation method in comparison with the vacuum pervaporation method. The feed coming into contact with the membrane vaporizes at the membrane surface. The generated vapor passes through pores of the membrane surface,

Table 4 Features of Thermopervaporation

		THERMOPERVAPORATION	PERVAPORATION
PRINCIPLE	MODEL		
	MECHANISM	EVAPORATION → PERMEATION	PERMEATION → EVAPORATION
	DRIVING FORCE	TEMPERATURE (STEAM PRESSURE) DIFFERENCE	PRESSURE DIFFERENCE
MEMBRANE	MATERIAL	HYDROPHOBIC	HYDROPHILIC
	STRUCTURE	POROUS	NON POROUS
OPERATION PRESSURE		NORMAL PRESSURE	EVACUATION
OPERATION TEMPERATURE		< BOILING POINT	ROOM TEMPERATURE



$\Delta P_{H_2O} = (P_{H_2O})_H - (P_{H_2O})_C$ VAPOR PRESSURE DIFFERENTIAL

ε	MEMBRANE POROSITY	D_M	DIFFUSION COEFFICIENT IN THE MEMBRANE
R	GAS CONSTANT	D_G	DIFFUSION COEFFICIENT OF GASEOUS PHASE
z	MEMBRANE THICKNESS	Z	GAP(TRANSFER DISTANCE)
δ	BOUNDARY LAYER THICKNESS		
T	TEMPERATURE		

Fig. 3 Membrane Separation Model of Thermopervaporation

diffuses to reach the cooling surface, where it is condensed. The driving force of the membrane permeate depends on the differential between the vapor content on the membrane surface and that on the cooling surface. The content differential is produced by the temperature differential of the feed and the permeate as shown in Fig.3, which illustrates a membrane separation model of thermopervaporation. Thermopervaporation, with its principles and features as stated above, offers wide applications such as the seawater desalting[4] [5], and the concentration of acids and alkalis[6], while research on the separation mechanism has continued.[7] [8] In the following sections, experiments on thermopervaporation are described.

3.2 Experimental Method

Fig.4 shows the flow of the experimental system. The membrane separation cell is composed of an evaporation room, a condensation room, and a cooling room in a layer structure using the hydrophobic membrane and the cooling surface. The hydrophobic membrane used is fabricated of PTFE (Nitto Electric Industrial Co., Ltd.) with a mean pore size of 0.05 ~ 2.0 μm and an effective membrane area of 148×10^{-2} m^2 (Table 5). The feed is heated to a specified temperature and sent to the evaporation room by the feed pump. The cooling water, which is cooled at a specified temperature, is circulated by the cooling water pump. The steam, which is

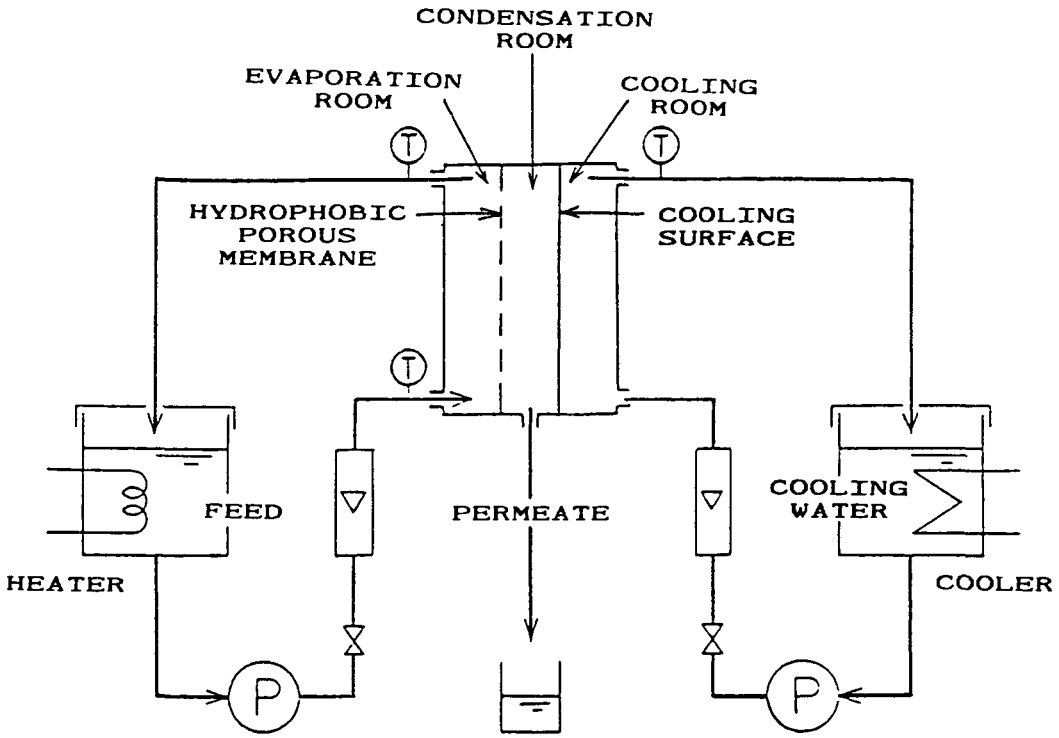


Fig. 4 Experimental Flow

Table 5 Porosity of PTFE Membrane (*)

PORE SIZE (μm)	MEMBRANE THICKNESS (μm)	POROSITY
2.00	83	0.84
0.15	70	0.72
0.05	89	0.64

(*) Fabricated by Nitto Electric Industrial Co., Ltd.

evaporated on the inner surface of the membrane in the evaporation room and passed through the membrane, is condensed on the cooling surface and then collected in a beaker for measurement.

The experimental study was performed under the following conditions for confirming the steam permeation mechanism of the thermopervaporation method and clarify factors affecting the membrane separation performance:

(1) Temperature of feed: 30 ~ 60 ℃
(2) Temperature of cooling water: 7 ℃
(3) Membrane pore size: 0.05 ~ 2.0 μm
(4) Gap between membrane and cooling surface: 0.8 ~ 25.3 mm

3.3 Experimental Results

3.3.1 Permeation performance and water quality

Fig.5 shows the separation characteristic of the NaCl aqueous solution and the water quality of obtained permeate represented by the electric conductivity. The permeate water flux Q is proportional to the saturated vapor pressure differential ΔP between the feed and the cooled permeate water. The water quality of the permeate is below 5 μS/cm, independent of the vapor pressure, compared with the feed electric conductivity of 49 mS/cm,

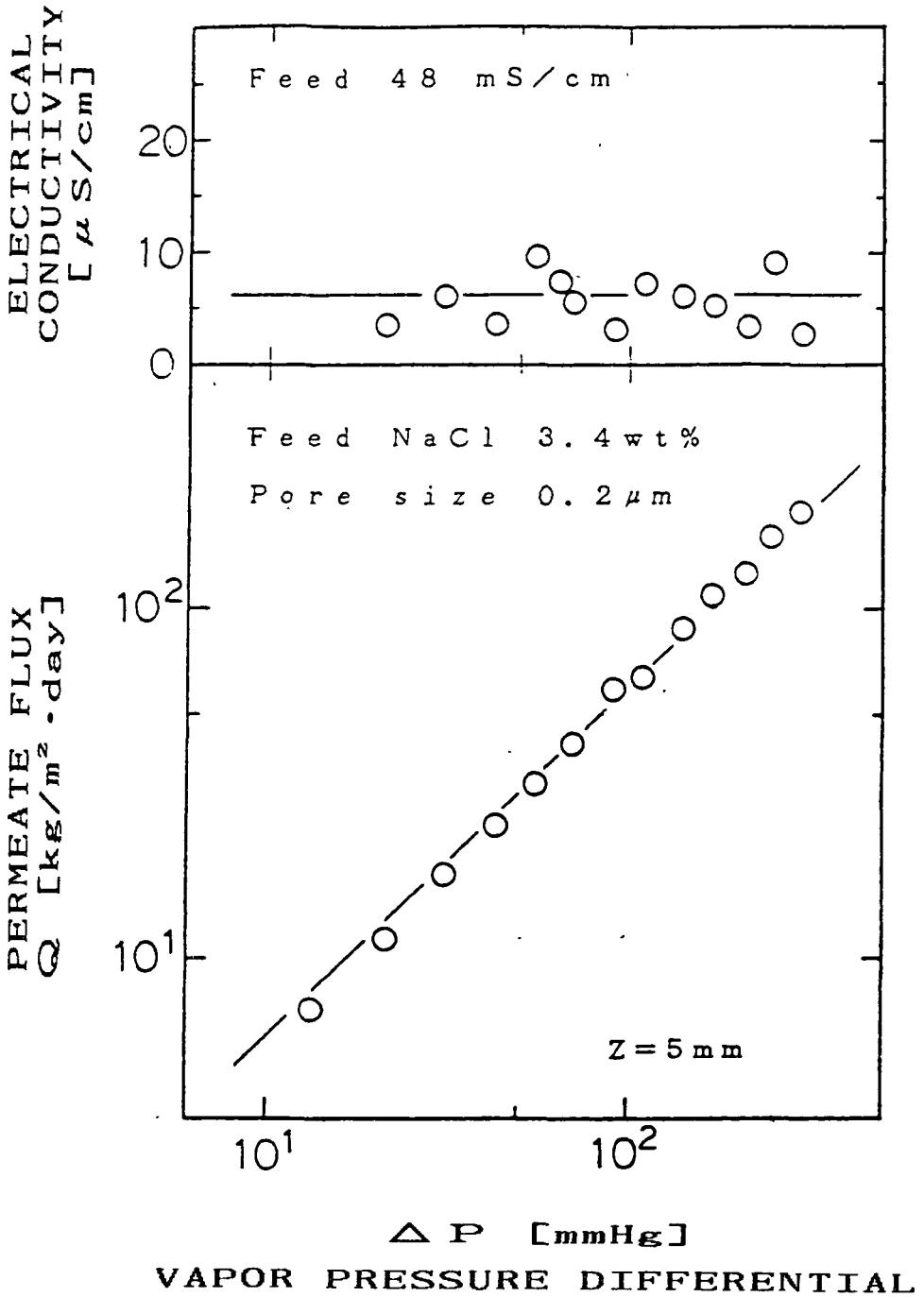


Fig. 5 Separation Characteristic of Water Quality of NaCl Aqueous Solution When Permeated through Thermopervaporation

the product water being ultrapure with the saline rejection of over 99.99 %.

3.3.2 Effect of gap between membrane and cooling surface

Fig.6 shows the relationship of the vapor pressure differential ΔP and the permeate flux Q, when the gap Z between the membrane and the cooling surface is varied. With any gap size the permeate flux is proportional to the vapor pressure differential with a tendency similar to that in Fig. 3: with gaps of less than 5.3 mm the permeate flux increases as the gap becomes small, and with gaps of more than 10.3 mm the permeate flux demonstrates almost no change. We have determined that the permeate mechanism changes with a gap around 5 mm.

3.3.3 Investigation of permeate mechanism

Using the experimental data in Fig.6, the relation of the gap Z and the permeation factor K was obtained as shown in Fig.7, where the permeation factor K is defined by Eq.(1).

$$K = \frac{Q}{\Delta P} \quad (kg/m^2 \cdot day \cdot mmHg) \qquad (1)$$

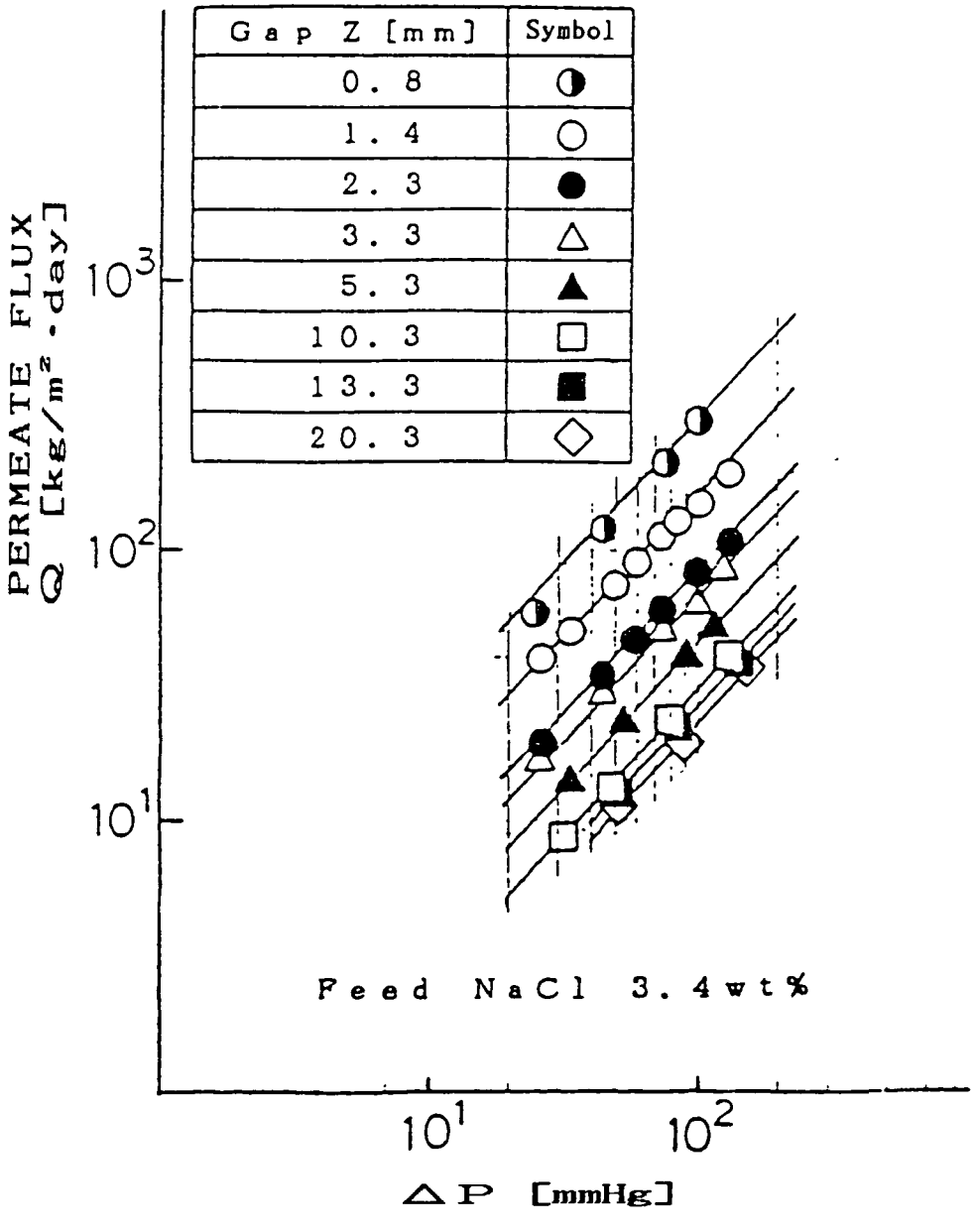


Fig. 6 Effect of Gap on Permeate Flux

(REMARK) D: Diffusion coefficient (m^2/day)

π: Total pressure (mmHg)

T: Mean temperature (°K)

$\overline{Pair}$: Logarithmic mean partial air pressure

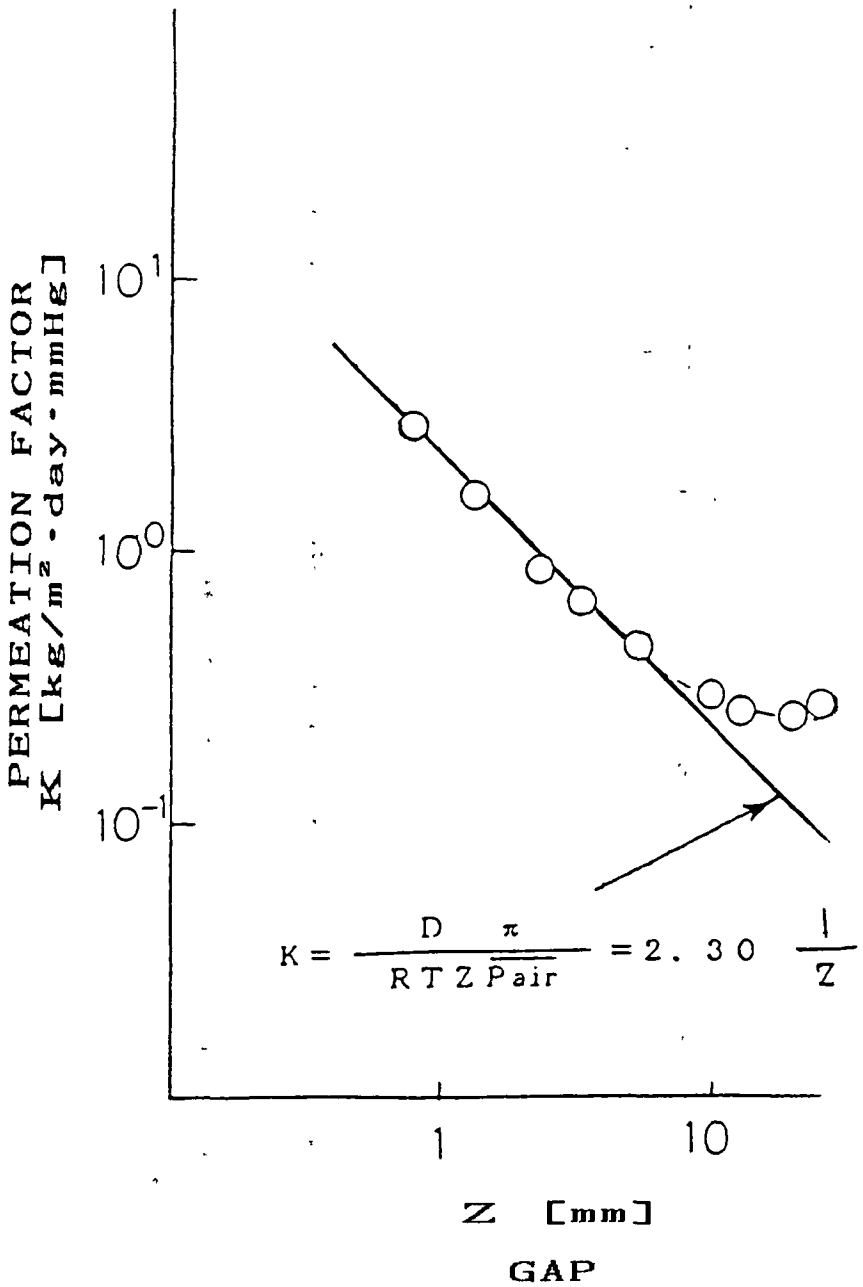


Fig. 7 Effect of GAP on Permeation Factor

As a result, with gaps below 5 mm the permeation factor is proportional to the minus first order of the gap, and the permeation factor shifts off the line of the minus first order and then becomes constant with gaps over 5 mm. In other words, the permeation factor is inversely proportional to gaps less than 5 mm but is not influenced with gaps more than 5 mm. From this, it is deduced that with gaps less than 5 mm, the permeate water flux depends on the steam diffusion in the condensation room, and with gaps more than 5 mm, on the natural convection produced in the condensation room to cause the vapor transfer.

With gaps below 5 mm, we computed the quantity of the steam diffusing in the condensation room by using the diffusion equation. Since air in the condensation room can be postulated to be stationary, we can treat it as unilateral diffusion.

The steam travel speed Q' can be represented as:

$$Q' = \frac{D \cdot \pi}{R\,T\,Z\,\overline{P_{air}}} \cdot \Delta P, \qquad (2)$$

where D is the diffusion coefficient (m^2/day), π the total pressure (mmHg), T the mean temperature (°K), and $\overline{P_{air}}$ the logarithmic mean partial air pressure (mmHg).

Consequently, the permeation factor K is expressed as :

$$K = \frac{Q'}{\Delta P} = \frac{D \cdot \pi}{R\,T\,Z\,\overline{Pair}} \qquad (3)$$

Using parameters under the present condition, Eq.(3) is simply modified into:

$$K = 2.30 \cdot \frac{1}{Z} \qquad (4)$$

In this modification, the temperature dependency of the diffusion coefficient D is obtained as Eq.(5) by plotting the results[9] of E.N. Fuller, et al.

$$D' = 1.4 \times 10^{-3} \cdot T' + 0.22, \qquad (5)$$

where D ′ is the diffusion coefficient (cm^2/sec), and T ′ the temperature (℃).

The solid line in Fig.5 shows the calculated relation (Eq.(4)) of the gap and the permeation factor. From this, we found that a close agreement between Eq.(4) and experimental results with gaps less than 5 mm and the permeation factor, namely the permeate water flux, agrees with the diffusing steam quantity in the condensation room.

From the results of the thermopervaporation experiment that was carried out on the ground as stated above, we can conclude that while the mass transfer of the steam is determined by the natural convection in the condensation room with gaps above 5 mm, it is determined by the steam diffusion speed in the condensation room with gaps less than 5 mm.

From this experimental data, the permeate flux Q is expressed as:

$$Q \propto \frac{\Delta P_{H_2O}}{RT\left(\underbrace{\frac{z}{D_M \cdot \varepsilon}}_{\text{MEMBRANE RESISTANCE}} + \underbrace{\frac{2\delta}{D_G}}_{\text{GASEOUS RESISTANCE}}\right)}, \qquad (6)$$

where $\Delta P_{H_2O} = (P_{H_2O})_H - (P_{H_2O})_C$ is the vapor pressure differential, R the gas constant, T the temperature, Z the membrane thickness, D_M the diffusion coefficient in the membrane, ε the membrane porosity, δ the boundary layer thickness, and D_G the diffusion coefficient of gaseous phase, as shown in Fig.3.

However, since it is said that the natural convection, in general, does not occur under zero gravity, the permeation dominated by the steam diffusion should be expected irrespective of the gap between

the membrane and the cooling surface in the condensation room. As a result, with gaps more than 5 mm, efficiency of the evaporation using the thermopervaporation method in space becomes lower than in the ground, because the permeation factor is inversely proportional to the gap as expressed in Eq.(4).

4. CONCLUSIONS

The restricted utility resources usable in the space station demand on compact hardware and low consumption power as well as reliability. If a shower system with low water utilization in the space station can be developed, compact and low power consuming equipment can be easily constructed.

The thermopervaporation method enables the use of low quality energy, such as exhausted heat from other equipment, as the evaporation energy. Thus, one possibility is to use exhausted heat from the gas recycle equipment which may be installed next to the water recycling equipment. The serial operation of each step of membrane processing, like interlock circuits, reduces the operating peak power, which is suitable for adhering to safety requirements.

Another water recycling system[10] to be applied to the Japan's experimental module will be used for life science experiments.

The system scale is similar to that presented here, but with a relatively different configuration due to the different constituents which contain no surfactants. The system configuration presented here is usable for Japan's experimental module.

An experimental study on thermopervaporation was made on the ground. In this experiment, the condensation room in the thermopervaporation cell is filled with air, but as an alternate application for space, the condensation room contains only steam to avoid mixture with air during the post process. Moreover, there are still many factors to be developed: a degasing process, thermal efficiency for low power consumption, reliable small pumps, separation of concentrate, cleaning of contaminated membrane, maintenance, etc.

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SUPERCRITICAL WASTE OXIDATION OF AQUEOUS WASTES

by
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ABSTRACT

For aqueous wastes containing 1 to 20 wt% organics, supercritical water oxidation is less costly than controlled incineration or activated carbon treatment and far more efficient than wet oxidation. Above the critical temperature (374°C) and pressure (218 atm) of water, organic materials and gases are completely miscible with water. In supercritical water oxidation, organics, air and water are brought together in a mixture at 250 atm and temperatures above 400°C. Organic oxidation is initiated spontaneously at these conditions. The heat of combustion is released within the fluid and results in a rise in temperature to 600-650°C. Under these conditions, organics are destroyed rapidly with efficiencies in excess of 99.999%. Heteroatoms are oxidized to acids, which can be precipitated out as salts by adding a base to the feed. Examples are given for process configurations to treat aqueous wastes with 10 and 2 wt% organics.

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INTRODUCTION

Aqueous wastes containing organic materials represent a major fraction of the total toxic and hazardous waste generated in the U.S. The predominant treatment methods have been deep-well injection and lagooning (including solar evaporation). These land-based disposal practices do not address ultimate destruction of the toxic components of the waste; consequently, there is increasing public concern with continued reliance on these techniques. Destruction methods, which usually cost an order of magnitude more than disposal methods, are based on oxidation of the organic content. For aqueous wastes, destruction methods include activated carbon treatment (with oxidative regeneration), incineration, wet oxidation and supercritical water oxidation.

For very dilute aqueous wastes (e.g., below 1% organic), activated carbon treatment is a viable alternative. Organics are desorbed and then oxidized during regeneration of the carbon. Since a significant fraction of the activated carbon is lost to oxidation during regeneration, fresh carbon must be added each cycle as make up. After-burners are used to destroy vapors that may result from partial oxidation during regeneration. The major cost of carbon treatment is the cost of regenerating the carbon and

providing the make up carbon. Since the cost per gallon is nearly proportional to the organic content, carbon treatment is usually not considered for wastes containing more than 1% organic.

Incineration, on the other hand, is usually restricted for economic reasons to relatively concentrated aqueous wastes. To attain high destruction efficiencies in treating toxic waste, incineration is conducted at high temperature (900-1100^{o}C) with relatively long residence time (e.g.,2 sec). With aqueous wastes, the heat required to bring the water component of the waste to these severe conditions is substantial. If the waste contains 25% organic or more, there is sufficient heating value in the waste to sustain the process. With decreasing organic content, the supplemental fuel required to satisfy the energy balance becomes a major cost. Thus, controlled incineration of aqueous wastes with less than 20% organic is only considered in extenuating circumstances.

In the range of concentrations of 1 to 20% organic, wet oxidation and supercritical water oxidation are far less costly than controlled incineration or activated carbon treatment. In wet oxidation of hazardous wastes, organics are oxidized in the aqueous phase at temperatures in the

range of 150 to 300°C and under pressures of 100 to 150 atm (1). In practice, residence times of 0.5 to 2 hr are required for removal of 50 to 95% of the initial COD. The residual organics are primarily innocuous aliphatic acids, which are by-products of oxidation of more complex molecules, and which are oxidized very slowly under wet oxidation conditions. Destruction of toxic organic chemicals (e.g., chlorophenols, nitrotoluenes) can be as high as 99.9%, but many materials are more resistant (e.g., chlorobenzenes and PCB's).

Although wet oxidation is more energy-efficient than incineration, the process suffers from a number of limitations. Under the conditions in which air and aqueous waste are mixed, the solubility of oxygen in water is considerably less than that required for complete oxidation. Thus, a two-phase mixture is present in the reactor. The reactor is an expensive item because it must operate at high temperature and high pressure with a relatively large volume to provide necessary residence time. Because the oxidation is not complete, the off-gas from the process can contain appreciable concentrations of volatile organics and may require additional treatment before release to the atmosphere.

SUPERCRITICAL WATER OXIDATION

Supercritical water oxidation of organics is an improvement upon wet oxidation and represents a breakthrough in enhanced efficiency and reduction in capital investment. The major advantages of operating supercritically are:

- o enhanced solubility of oxygen and air in water, which eliminates two-phase flow;
- o rapid oxidation of organics, which approaches adiabatic conditions as well as high outlet temperatures with very short residence times;
- o complete oxidation of organics, which eliminates the need for auxiliary off-gas processing;
- o removal of inorganic constituents, which precipitate out of the reactor effluent at temperatures above 450^{o}C; and
- o recovery of the heat of combustion in the form of supercritical water, which can be a source of high-temperature process heat or used to generate power in supercritical turbines.

These advantages arise primarily from the unusual properties exhibited by water under supercritical conditions.

The Properties of Supercritical Water

Above the critical temperature and pressure, the properties of water are quite different from that of the

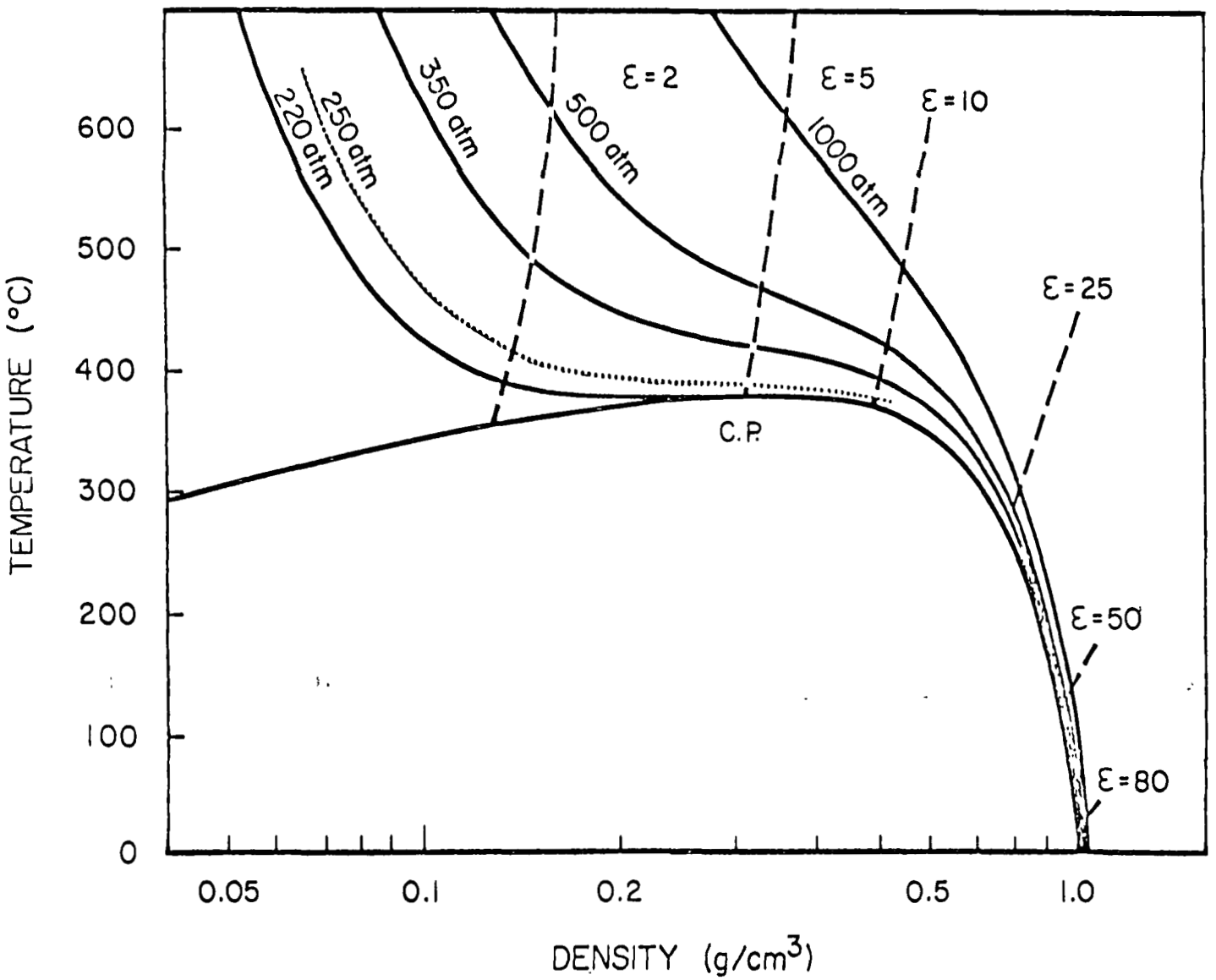


Figure 1

Temperature-density diagram for water with isobars (solid lines) and constant dielectric constant (dashed lines)

normal liquid or atmospheric steam. For example, organic substances are completely soluble (i.e., miscible in all proportions) in water under some supercritical conditions, while salts are almost insoluble under other supercritical conditions. These solubility characteristics are strongly dependent upon density.

A temperature-density diagram is shown in Fig. 1. The critical point (C.P.), which lies on the vapor-liquid saturation dome, occurs at 374°C and 0.3 g/cm^3. The supercritical region lies above 374°C and to the right of the 220 isobar. Near the critical point (e.g., between 300°C to 450°C and densities from 0.2 to 0.7 g/cm^3), the density varies very rapidly with relatively small changes in temperature at constant pressure.

Insight into the structure of the aqueous fluid in this region has been obtained from measurements of the static dielectric constant, which are also shown in Fig. 1 (2,3). The dielectric constants of some common solvents are given, for comparison, in Table 1.

The dielectric constant is a measure of the degree of molecular association. Normal liquid water has an ε of 80, largely as a result of strong hydrogen bonding. The

TABLE 1. DIELECTRIC CONSTANTS OF SOME COMMON SOLVENTS

Carbon Dioxide	1.60
n-Hexane	1.89
Benzene	2.28
Ethyl ether	4.34
Ethyl acetate	6.02
Benzyl alcohol	13.1
Ammonia	16.9
Isopropanol	18.3
Acetone	20.7
Ethanol	24.3
Methanol	32.6
Ethylene glycol	37.
Formic acid	58.

dielectric constant of the saturated liquid decreases rapidly with increasing temperature, even though the density falls slowly. Since hydrogen bonding forces are strong only when molecules are in close proximity, small increases in density parallel relatively large decreases in short-range order resulting in a rapid decline in ε. At 130°C (ρ = 0.9 g/cm^3), the dielectric constant is about 50, which is near that of formic acid; at 260°C (ρ = 0.8 g/cm^3), the ε of 25 is similar to that of ethanol.

As density decreases further, the dielectric constant becomes less dependent upon temperature (i.e., the lines of constant ε in Fig. 1 become almost vertical). At the critical point, the dielectric constant is 5. Ramon spectra of HDO in this region indicate little, if any, residual

hydrogen bonding (4). The major contribution to ε is now molecular association due to dipole-dipole interactions, which gradually decrease with increasing void volume.

Above 300°C, the temperature-density relationship becomes pressure-dependent (see Fig. 1). We shall follow the 250 atm isobar (which is a convenient pressure for supercritical operations). At 300°C, the ε of about 15 is similar to that of benzyl alcohol. Proceeding above 300°C along the 250 isobar, we see ε dropping to 10 at 400°C, 5 at 420°C and 2 at 490°C. Through the two hundred degree rise from 300 to 500°C, the dielectric constant varies from that of moderately polar to non-polar organic solvents.

While dielectric constant is not the sole determinant of solubility behavior, the solvent power of water for organics is consistent with the variation of ε, as described above. Benzene solubility in water is a good example (5,6). At 25°C, benzene is sparingly soluble in water (0.07 wt%). At 260°C, the solubility is about 7 to 8 wt% and fairly independent of pressure. At 287°C, the solubility is somewhat pressure dependent, with a maximum of 28 wt% at 200 to 250 atm. In this pressure range, the solubility rises to 35 wt% at 295°C; at 300°C, the critical point of the benzene-water mixture is surpassed.

When the mixture becomes supercritical, by definition, there is only a single phase. Thus, the components are miscible in all proportions.

Other hydrocarbons exhibit similar solubility behavior. Aliphatic hydrocarbons are somewhat less soluble in water at comparable temperatures. Thus, higher temperatures are required to reach the same solubility as that of benzene. Binary mixtures of pentane-water and heptane-water become supercritical (and, therefore, completely miscible) at about 350°C (5).

The locus of critical points of binary mixtures containing water have been reported for a variety of organic substances (e.g., ethane, ethylene, n-butane, 1,3,5 trimethylbenzene, naphthalene) and gases (nitrogen, carbon dioxide, ammonia, hydrogen, argon). Except for argon, all of the compounds studied to date are completely miscible with water above 374°C at 250 atm. Argon is completely miscible above 390°C.

For densities less than 0.7 g/cm^3, the solubility of inorganic salts in water is as unusual as that of organics. At 250 atm, the solubilities of salts reach a maximum at 300 to 450°C. Beyond the maximum, the solubilities drop very

rapidly with increasing temperature. For example, NaCl solubility is about 40 wt% at 300°C and about 100 ppm at 450°C; $CaCl_2$ has a maximum solubility of 70 wt% at subcritical temperatures, which drops to 10 ppm at 500°C (7). Given the fact that the dielectric constant of water is about 2 at 490°C and 250 atm (see Fig. 1), it is not surprising that inorganics are practically insoluble.

Coincident with the loss of solvating power for inorganic salts, supercritical water also loses the ability to dissociate salts. For example, the dissociation constant of NaCl at 400-500°C and densities in the range of 0.35 is of the order of 10^{-4}. Thus, strong electrolytes become weak electrolytes in supercritical water.

The properties of water, as a function of temperature, are summarized in Fig. 2. We see that water goes through a complete reversal in solubility behavior toward organic and inorganic substances through the temperature range of 350-450°C. Below this range, the pattern is similar to normal liquid water: low organic and high inorganic solubility. Within the range, there is high solubility of both organic and inorganic substances. Above this range, inorganic salts are practically insoluble, and organic substances are completely miscible.

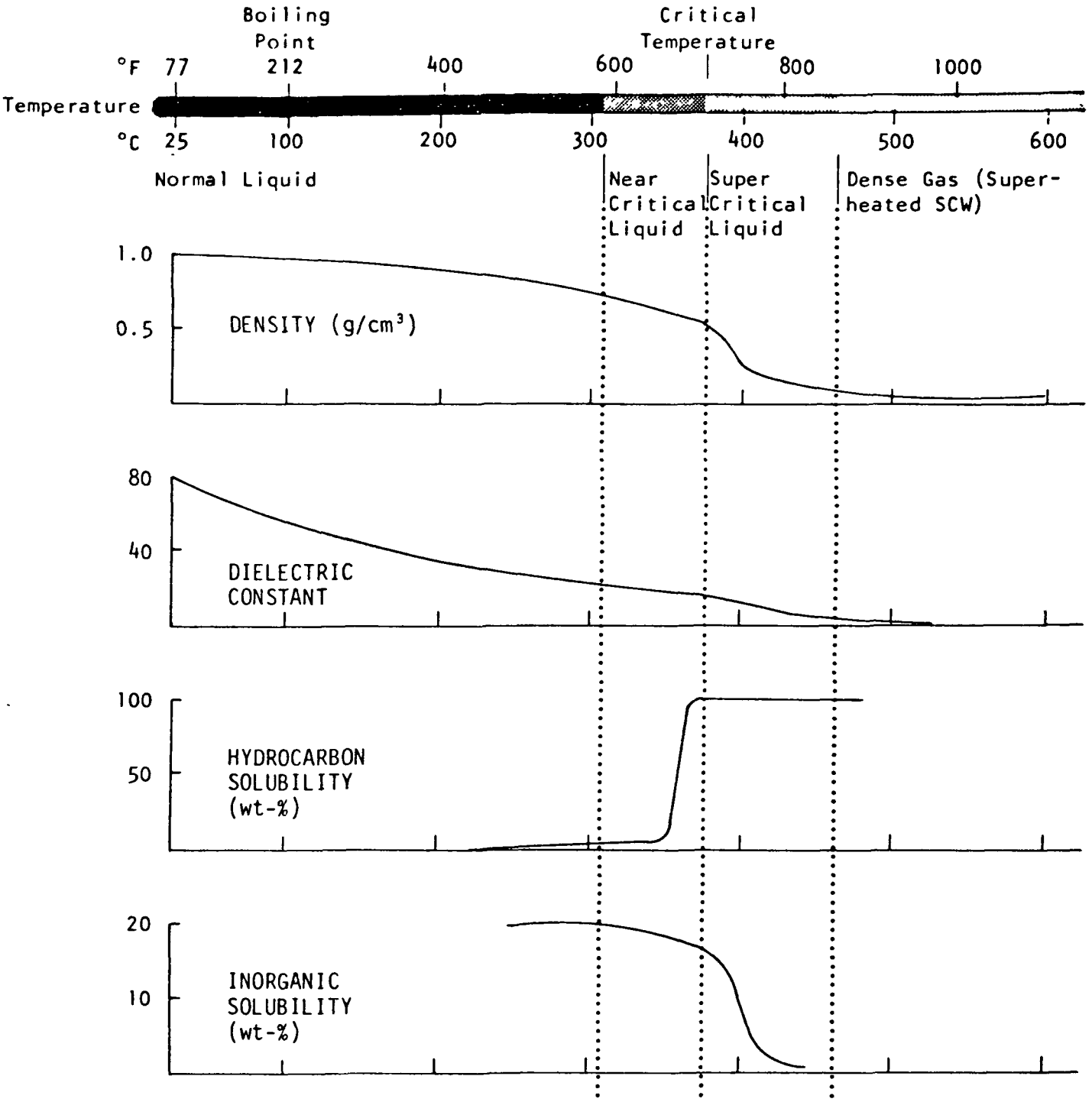


Figure 2

Properties of water at 250 atmospheres

The Effect of Temperature on Destruction Efficiency

Over the past five years, a number of studies of SCW oxidation have been conducted using a bench scale unit, which has been described previously (8,9). Reported here are results of a study of the effect of temperature on destruction efficiency. The results, shown in Table 2, are striking examples, yet quite typical of other results that have been obtained. The feed material for the tests of Table 2 were mixtures of 2,4-nitrotoluene in methylethyl ketone. All experiments were run with a residence time of 20 to 30 sec. Each column of Table 2 represents the average values of the two runs.

At the four temperatures of 404, 457, 513 and 574^{o}C, the organic carbon destruction efficiencies were 92.5, 99.8, 99.93 and 99.998%, respectively. These results correspond to an increase in rate of over 3 orders in magnitude in the temperature range of 404 to 574^{o}C. There is reason to believe that these results are limited not by reaction kinetics but by the rate of mixing of reactants. Rates of almost two orders of magnitude higher than the highest of Table 2 were observed when operating with a new feed introduction device at 630^{o}C (i.e., destruction efficiencies were greater than 99.999% at residence times as low as 0.6 sec).

TABLE 2

THE EFFECT OF TEMPERATURE ON DESTRUCTION EFFICIENCY; OXIDATION OF DINITROTOLUENE

Run No.	44/45B	42/43	39/40	69/70A
Feed Material	3% DNT/ MEK	3% DNT/ MEK	3% DNT/ MEK	12% DNT/ MEK
Temperature (°C)	404	457	513	574
Carbon Analysis				
Organic Carbon In (ppm)	9,540	9,650	9,750	17,530
Organic Carbon Out (ppm)	712	15.7	6.9	0.4
Destruction Efficiency (%)	92.5	99.8	99.93	99.998
Total Carbon Recovered (%)	99.9	98.6	100.6	106.1
Nitrogen Analysis				
Organic Nitrogen In (ppm)	64	65	65	511
Nitrogen Out-Liquid (%)	58.7[a]	10.6[a]	2.2[a]	6.4[b]
Nitrogen Out-Gas (%)	NA	23.1[c]	23.1[c]	94.4
Total Nitrogen Recovered (%)	58.7	33.7	25.3	100.8
Gas Composition (mol %)				
O_2	16.96	11.88	15.04	15.08
CO_2	70.40	88.85	81.24	84.80
CH_4	0.10	0.06	0.18	ND
H_2	1.68	0.08	0.04	ND
CO	9.63	0.29	0.35	ND
N_2	0.10	0.06	0.06	0.41
N_2O	NA	NA	NA	0.57
NO	NA	NA	NA	ND
Liquid Products Containing Nitrogen (ppm)				
Dinitrobenzene	170.4	0.187	0.0066	<1 ppb
Dinitrophenol	6.1	0.002	0.0046	<1 ppb
Dinitrotoluene	60.5	0.535	0.0087	<1 ppb
Dinotrocresol	1.2	0.024	0.0018	<1 ppb

[a] [b] [c] Primarily organic nitrogen in liquid products
Primarily nitrate and nitrite nitrogen
Molecular nitrogen

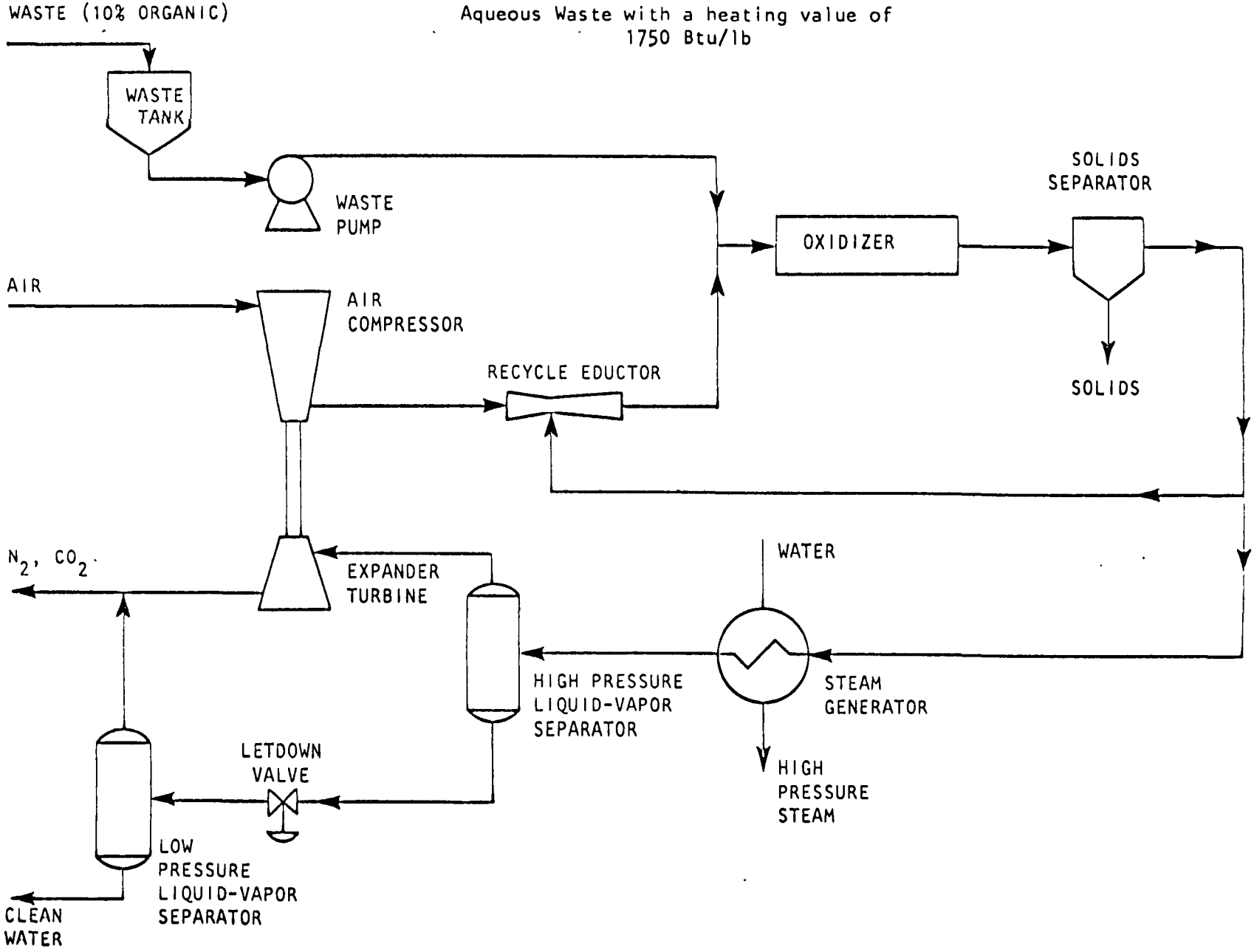
It is interesting to note the fate of organic nitrogen in the dinitrotoluene oxidation tests. At 574°C, the last column of Table 2, 94% of the nitrogen in the feed is recovered as N_2 and N_2O in the gaseous effluent and 6% is recovered as nitrate and nitrite ions in the liquid effluent. No NO, NO_2 or NH_3 was found in the gas phase nor was NH_3 found in the liquid phase.

In the other three columns of Table 2, the nitrogen material balance closure was poor because we did not analyze for N_2O in the gas phase and NH_3 in the liquid phase. In other experiments with dinitrotoluene, ammonia and amines were found at 400 to 500°C while N_2O is the major product at 500 to 550°C.

In no case were NO and NO_2 formed as products of oxidation of nitrogen compounds in SCW oxidation. At 600 to 650°C, N_2 and N_2O are the major products, even when the feed is primarily ammonia nitrogen. The N_2O component can be readily decomposed catalytically to N_2 and O_2 and, thus, SCW oxidation of nitrogen-containing organics is far less damaging to the environment than high temperature incineration.

Figure 3

Process Schematic for Oxidation of an Aqueous Waste with a heating value of 1750 Btu/lb



SCWO for Treatment of Aqueous Wastes

Supercritical water oxidation can be applied to wastes with a wide range of organic concentration. A schematic flowsheet for a process for treating an aqueous waste containing 10 wt% organic is given in Fig. 3. This process consists of the following steps:

1. The waste, as either an aqueous solution or a slurry, is pressurized and delivered to the oxidizer inlet. It is heated to supercritical conditions by direct mixing with recycled reactor effluent.
2. Oxygen is supplied in the form of compressed air, which is used as the motive fluid in an eductor to provide recycle of a portion of the reactor effluent. This inlet mixture is then a homogeneous phase of air, organics and supercritical water.
3. The organics are oxidized in a controlled but rapid reaction. The short residence times required allow adiabatic operation of the oxidizer. The heat released by combustion of readily oxidized components is sufficient to raise the fluid phase to temperatures at which all organics are oxidized rapidly.
4. The effluent from the oxidizer is fed to a cyclone. The solubility of inorganics is extremely

low at the reactor effluent temperatures. Inorganic salts that are originally present in the feed or which form in the combustion reactions precipitate out of the fluid phase in the oxidizer and are separated here.

5. The fluid effluent of the solid separator is a mixture of H_2O, N_2, and CO_2. A portion of this is recycled through the eductor to provide supercritical conditions at the oxidizer inlet.
6. The remainder of the effluent is available as a high temperature, high pressure fluid for energy recovery. This stream is cooled to a subcritical temperature in a heat exchanger which serves to generate low pressure or high pressure steam.
7. Now at a subcritical temperature, the mixture has formed two phases and enters a high pressure liquid-vapor separator. Practically all of the N_2 and most of the CO_2 leaves with the gas stream. The liquid consists of water with an appreciable amount of dissolved CO_2.
8. The gas stream can then be expanded through a turbine to extract the available energy as power. A portion of the power is used for compression of the inlet air.
9. The liquid from the high pressure separator is

depressurized and fed to a low pressure separator. The vapor stream is primarily CO_2 which is vented with the gas turbine effluent. The liquid stream is clean water.

The destruction efficiency (defined herein as the conversion of organic materials in the feed to final oxidation products of CO_2, H_2O and acids or oxyacids of heteroatoms and metals) is a function of reactor temperature and residence time. It has been found that a reactor effluent temperature in the range of 600 to 650°C and residence time of 5 sec are sufficient for a destruction efficiency of more than 99.999%. Higher temperatures could be used to reduce the residence time. However, at 5 sec residence time, the reactor cost is a small fraction of total capital cost and, therefore, there is not much incentive to try to reduce reactor volume by operating above 650°C.

Increasing either temperature or residence time will result in increased destruction efficiency. The oxidation kinetics appear to be approximately first order in organic concentration. Thus, in theory, doubling the reactor length and, thereby, doubling the residence time from 5 to 10 sec should result in doubling the logarithm of destruction

efficiency; in other words, 99.999% should become 99.999999999%! [Of course, the analytical techniques required to document such high destruction efficiencies are not available.]

For the process configuration illustrated in Fig. 3, the reactor exit temperature is a direct function of the heating value of the feed. To attain a temperature of 600 to 650°C, the waste should contain about 1,750 Btu/lb or 4,050 J/g, which is the heating value of an aqueous solution of about 10 wt% benzene (heat of combustion of 17,500 Btu/lb or 40.5 kJ/g) or 14 wt% ethanol (12,800 Btu/lb or 29.7 kJ/g). If the waste is more concentrated or otherwise has a higher heating value, it could be blended with more dilute waste or, if unavailable, with water. In the latter case, the added water would be recovered from the process effluent in a form which is pure enough to be used as process water in most applications.

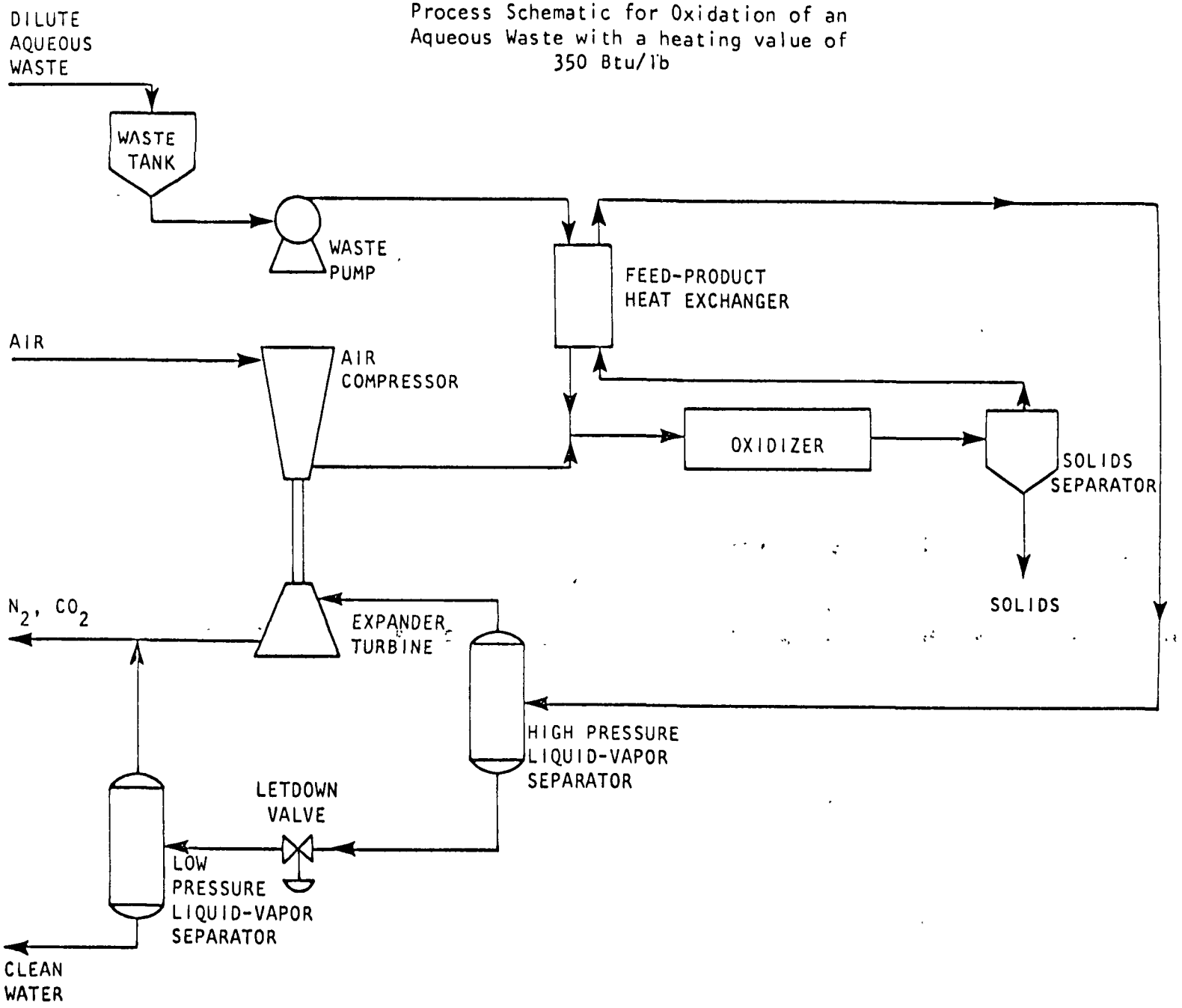
The energy released by combustion is contained within the reactor effluent as thermal energy. As shown in Fig. 3, it could be recovered as heat in the steam generator and/or power from the expansion turbine. The energetics of the SCW oxidation process are such that the amount of power available for recovery is substantially more than that required

to compress the air and waste. The overall process is somewhat analogous to a gas turbine power cycle. However, many applications require systems which are small (by chemical process industry standards) and capital-intensive. In those cases, power recovery cannot be justified on economics and, thus, the heat of combustion and the energy input for air compression are simply recovered as steam.

For wastes with heating values below 1,750 Btu/lb, auxiliary fuel could be added to make up the required heating value. The fuel cost can be appreciable when treating a very dilute waste. In such cases, it is more economical to use a regenerative heat exchanger rather than a steam generator, as shown in Fig. 4. This process configuration differs from that of Fig. 3 by addition of a feed-effluent heat exchanger and deletion of steam generator and eductor. In this manner, the minimum heating value of the feed for autogenic operation is 350 Btu/lb (800 J/g), which is a concentration of 2 wt% benzene-equivalent. For the same heat release rate, the process of Fig. 4 can treat five times as much waste (at 2 wt%) as that of the process of Fig. 3 (at 10 wt%). This increased waste volume more than compensates for the higher capital cost due to the regenerative heat exchanger.

Figure 4

Process Schematic for Oxidation of an Aqueous Waste with a heating value of 350 Btu/lb



Advantages of Supercritical Water Oxidation

As a waste destruction process, SCW oxidation has several advantages over conventional processes. The chemical reactions that occur are carried out in a closed system, making it possible to maintain total physical control of waste materials from storage, through the oxidation process, to the eventual discharge of the products of combustion. This feature provides positive assurance of environmental protection. In addition, bench-scale results have demonstrated essentially complete combustion of chemically stable hazardous and toxic materials. The process can be adapted to a wide range of feed mixtures and scales of operation. Systems can be designed as skid--mounted, transportable units and as larger scale stationary units. The process is capable of generating all the power required for air compression and feed pumping and, thus, can have no net energy requirement for system operation.

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AIRBORNE TRACE CONTAMINANTS OF POSSIBLE INTEREST IN CELSS

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ABSTRACT

One design goal of CELSS for long duration space missions is to maintain an atmosphere which is healthy for all the desirable biological species and not deleterious to any of the mechanical components in that atmosphere. CELSS design must take into account the interactions of at least six major components; (1) humans and animals, (2) higher plants, (3) microalgae, (4) bacteria and fungi, (5) the waste processing system, and (6) other mechanical systems. Each of these major components can be both a source and a target of airborne trace contaminants in a CELSS. A range of possible airborne trace contaminants is discussed within a chemical classification scheme. These contaminants are analyzed with respect to their probable sources among the six major components and their potential effects on those components. Data on airborne chemical contaminants detected in shuttle missions is presented along with this analysis. The observed concentrations of several classes of compounds, including hydrocarbons, halocarbons, halosilanes, amines and nitrogen oxides, are considered with respect to the problems which they present to CELSS.

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INTRODUCTION

The ultimate objective of the CELSS research program is to construct a life support system for long duration space missions capable of providing food, water and oxygen and of recycling wastes. The major problem in developing such a system can be viewed as achieving mass balance between subsystems by controlling flows of matter, energy and information. Before that ultimate goal is reached, a preliminary objective for the CELSS research program would be to construct a life support system for long duration space missions capable of maintaining an atmosphere healthy for all the desirable biological species and not deleterious to any of the mechanical components. In order to approach this preliminary objective it will be necessary to predict, identify and control the trace contaminants which any CELSS components could release that would be harmful to any other component.

The inclusion of higher plants or microalgae in a CELSS system almost certainly guarantees that there will be problems with trace contamination. That is because many species of plants and algae have evolved allelopathic responses; they excrete or emit substances which have the effect of lessening the competition for local resources by other individuals (J. Friedman and G. R. Waller, 1985, TIBS 10, 47-50). Early experiments with closed systems including both plants and algae revealed that the algae released a volatile substance that could seriously damage or kill the plants (M. M. Korotayeu, et al., 1964, Problemy Kosmicheskoy Biologii 3, 204), a result that could be attributed to allelopathy and autotoxicity in the the algae (R. Pratt, et al., 1944, Science 99, 351-352).

At a CELSS workshop conducted in September 1984 some concern was expressed that the problems of controlling of trace contamination deserved more study and research. When data on trace contaminant detection, identification and control on space shuttle missions became available, it seemed reasonable to approach the study of potential trace contamination problems in CELSS by examining actual trace contamination problems on the shuttle.

APPROACH

Identification of Major Subsystems

There are many ways in which the components of a life support system can be grouped into subsystems depending on the objectives of the analysis. For the purposes of analyzing sources and targets of airborne trace contaminants, six major subsystems of a CELSS are identified. These subsystems are referred to in Table 1.

- H -- Humans and possibly other animal species consume O_2, H_2O, food, energy and information and generate CO_2 and waste.
- P -- Higher plants consume CO_2, H_2O and energy, and generate O_2, food and waste.
- A -- Microalgae also consume CO_2, H_2O and energy, and generate O_2, food and waste.
- B -- Bacteria, virus and fungi will in most cases themselves be considered as potential airborne trace contaminants. However, some may be intentionally introduced as sources of food, as aids in processing either food or waste, or as controls to inhibit the growth of other more harmful bacteria and fungi.
- M -- Machines, including specifically all the surfaces enclosing and connecting the other subsystems, may potentially outgas, degrade or react to release volatile contaminants. The machine subsystem may also accumulate and concentrate volatile contaminants released by other subsystems.
- W -- The waste processing system considered separately from the machine subsystem includes the waste products of the biological subsystems and all the intermediate materials of that processing. Inherently this subsystem would be a major source of volatile contamination should it malfunction. It could also be a highly vulnerable target for unanticipated volatile contaminants.

The chemical elements involved in the mass balance problem can be grouped according to their biological significance. Of primary importance are C, O, H, and N; of lesser importance are P and S; and of minor importance are Na, K, Mg, Ca, Fe, Cu, Zn, Cl and maybe a few others such as Br, I, Si, Mo, V and W. The other 84 chemical elements will probably not enter the mass balance problem but may be of interest as trace contaminants. The major chemical species involved in the mass balance problem are O_2, CO_2, H_2O, food (CHNO) and to a lesser extent N_2.

An analysis of airborne trace contaminants will focus on minor components in this mass balance problem: those very minor components which might be missed even if the mass equations were balanced to the fourth or fifth significant figure, but components which could nonetheless have a serious detrimental effect on at least one major subsystem. Drawing an analogy from toxicology, the concentration dependence of the detrimental effects of such trace contaminants can be characterized in two ways.

(1) A contaminant will damage a subsystem when it persists above a minimum atmospheric concentration for a period on the order of days: a condition analogous to chronic toxicity.

(2) A contaminant will damage a subsystem when it is released in such quantities that it exceeds a minimum atmospheric concentration: a condition analogous to acute toxicity.

The airborne trace contaminants are defined to be those components which are gases or can be volatilized at normal temperatures and pressures, or which can be transported on normal atmospheric currents. Table 1 is a listing of classes of chemical compounds which are potential airborne trace contaminants in a CELSS. The table includes an evaluation of which major subsystems could be sources and which could be targets for each class of compounds. It would be appropriate to offer a

few comments on how these airborne trace contaminants might be detected and eliminated in a CELSS.

Methods of Detection

Only two methods of airborne trace contaminant analysis will be generally applicable, IR and GC/MS. Visible and UV light monitoring of airborne bacteria, fungi and their spores will be important. Monitoring of toxic, volatile trace contaminants should be done at a frequency proportional to their toxicity, and that requirement well mean that monitoring will have to be done in real time, on board the space craft. In addition some compounds might be monitored as indicators of stress on particular subsystems, for example ethylene, ethane and methane could be monitored to indicate stress on the higher plants.

Methods of Elimination

In order to remove airborne trace contaminants it will be necessary to collect and chemically transform them selectively. In a completely closed system regeneration of the constituent elements of trace contaminants would be necessary. For incompletely closed systems chemical transformation of trace contaminants is not necessary only insofar as their loss does not significantly affect the mass balance. Storage of collected contaminants would be inherently hazardous, but jettisoning of that material might be unacceptable because it would compromise experiments or procedures dependent on vacuum conditions near the space craft.

OBSERVATIONS

Table 2 is a listing of the airborne trace contaminants which have been observed on the shuttle missions to date. They are grouped according to the classification scheme in Table 1. These trace contaminants were collected on space shuttle missions either in gas sample bottles manually operated by the astronauts, or in activated charcoal filters or canisters of lithium hydroxide which were components of the environmental control system. The contaminants were later identified in ground laboratories by GC/MS. A few particular contaminants which have been observed, and some which have not been observed, require special comment.

Methane is assumed to have a biological origin, the humans and their associated bacteria. It has a low toxicity, so it not dangerous in that respect. However it is not readily adsorbed by charcoal and in some simulations its atmospheric concentration has risen high enough to present a fire hazard.

Dimethyl benzene (toluene), which is carcinogenic, has been observed as an airborne contaminant on every shuttle mission. On one shuttle mission the amount of toluene in the gas bottle samples indicated that the spacecraft maximum allowable concentration (SMAC) had been exceeded. Although the source of the toluene was not positively identified, it is thought that an astronaut had taken gas bottle samples while holding an uncapped marking pen.

A large number of silicone compounds have been observed in fairly high concentrations. These compounds are notoriously difficult to remove once they have been applied to surfaces. Contamination from them is generally not considered to pose a problem because of their low toxicity to humans. Two silicone contaminants which are toxic and have been observed are propylfluorosilane and propyldifluorosilane.

On several occasions relatively large amounts of bromotrifluoromethane have been observed. This mildly toxic compound is released by leaking fire extinguishers. Twenty-three other halocarbons have also been observed, some of them at relatively high concentrations. Most of these halocarbons are thought to arise from the outgassing of residual solvents used for degreasing and removing excess silicones.

Dichloroethyne (C_2Cl_2) is an extremely toxic compound not known to be a contaminant or an outgassing product of any substance present in the shuttle. However it can be produced by dehydrohalogenation of trichloroethene (C_2HCl_3) in strong base. This latter compound is commonly used as an industrial solvent, for degreasing and dry cleaning. Undoubtedly it has been used inside the shuttle because it has also been trapped and detected in the activated charcoal filters. However, the activated charcoal filters did not collect all the trichloroethene. Some of it must have passed through the charcoal to the lithium hydroxide canisters which are next in the air flow path and are used to scrub carbon dioxide from the air. The trichloroethene reacted in the lithium hydroxide canisters to form dichloroethyne which passed back out into the cabin atmosphere. It was then picked up in a subsequent pass through the activated charcoal filters.

The shuttle missions have had some variability in the number of airborne bacteria; between 100 and 350 culture forming units per cubic meter have been observed including both aerobes and anaerobes. The spores of one major fungus, Candida albicans, have been observed, but fungal growth does not appear to be a problem on the shuttle. If there is a problem, it might not be evident until longer duration missions are undertaken. On the earth, gravity acts like a filter limiting the propagation of airborne fungal spores. In a low gravity environment the spores could be expected to propagate more readily.

Among the compounds which have not been observed are hydrogen cyanide and benzonitrile, although they have been observed in tests involving the pyrolysis of electrical insulation. Ethene (ethylene) which has diverse and striking effects on higher plants at concentrations as low as 5 ppb, has also not been observed.

DISCUSSION

The trapping of dichloroethyne in the shuttle illustrates particularly well why great care is required in studying the interactions of CELSS subsystems. The way in which it must have been generated in the space shuttle clearly illustrates how two subsystems can interact to produce hazardous trace contaminants. The possibility that chloroethyne and dichloroethyne could be generated in that way had been pointed out by R. Saunders (1967, Arch. Environ. Health 14, 380-384) in reports of very early space flight simulations.

Another lesson provided by the odyssey of chloroethyne in the space shuttle is that the activated charcoal filters have a finite capacity that can be overwhelmed with unpleasant consequences. In order to be effective, activated charcoal filters must be changed on a regular basis. For long duration space missions this means that large numbers of spent filters must either be jettisoned, stored and returned to earth, or recharged with the discharged contaminants being either evacuated or converted by the waste processing system. Only the last alternative can be considered desirable in a CELSS. The problems with activated charcoal filters can be expected to become even more critical when higher plants or microalgae are included in a CELSS because of the relatively large amounts of volatile organic compounds which they produce.

From the available trace contamination data, the class of compounds posing the most immediate danger is the haloalkanes. They meet both criteria for the definition for chronic contaminants; they are not completely adsorbed or retained by the activated charcoal filters, so they tend to persist in the shuttle atmosphere, and as a class the haloalkanes are chronic toxins to humans. It must be hoped that steps will be taken to limit the use of haloalkanes on the shuttle, on the space station and certainly in a CELSS.

Another class of compounds which presents a large potential danger as trace contaminants is the siloxanes. These compounds, like the haloalkanes, tend to be chemically inert, but unlike the haloalkanes, they are mostly nontoxic to humans. The low molecular weight compounds are easily volatilized, not readily adsorbed by activated charcoal and tend to persist in spacecraft atmospheres. They have been observed as trace contaminants in every spacecraft since the Mercury capsule. Unfortunately these compounds do present a number of problems. First, their tenacious persistence on surfaces leads to the heavy use of haloalkanes in efforts to remove them. Second, the alterations in the surface properties of silicate glass which they can produce at even low concentrations might seriously compromise cell tissue culture work envisioned for future shuttle experiments. Third, no research has been done on the long term effects of low atmospheric concentrations of these compounds on plants. Fourth, for some experimental waste processing systems, it is possible that these compounds could be difficult to handle.

Another potential source of trace contamination is aging synthetic polymers. With time all synthetic polymers suffer depolymerization which is usually accompanied by the release of a large number of gaseous compounds. Such polymers have been used extensively in the shuttle and presumeably they will continue to be used in the construction of spacecraft for many years. The aging of synthetic polymers is accelerated by exposure to light, ionizing radiation, trace contaminants, water and oxygen. In spacecraft the aging processs could be worse because of high energy radiation and atmospheres of enriched oxygen. (Oxygen radical species are created in proportion to the partial pressure of oxygen, but in pure atmospheres there is less probability for destructive collisions so the radical species can reach higher concentrations.) In a CELSS including either higher plants or microalgae, the exposure to light and humidity can be expected to accelerate the aging of polymers used in the growing chambers.

An enumeration of the airborne trace contaminants observed on the shuttle is useful in determining what contamination problems can be dealt with in the current life support system, a system consisting of just human, bacterial and machine subsystems. For a CELSS including a waste processing system and either higher plants or algae, the list of trace contaminants will certainly include even more compounds from the classes listed in Table 1. With a greater variety of trace contaminants present, the probability of synergistic effects also becomes greater; several trace contaminants could react to form an even more hazardous compound, a target substance could be more susceptible to a combination of contaminants, or a contaminant could catalyze a reaction which released more contaminants. Fortunately, with more trace contaminants present, there is also an increased probability that some of them could react and become neutralized, but that is the sort of good fortune that should not be relied upon. In a CELSS it will be very important to be able to identify trace contaminants rapidly and to have a data base of chemical properties and reactions which can be used to predict the consequences of contamination.

ACKNOWLEDGMENTS

I am grateful to Ms. Mary Schwartz for graciously providing me with data on airborne trace contaminants on space shuttle missions and to Ms. Sofia Oldmark for diligently and patiently entering that data into the computer.

TABLE 1. Potential Airborne Trace Contaminants, Sources and Targets in CELSS

G indicates that the compound is generated by the listed subsystems, E indicates that the compound has an effect on the listed subsystems, and H, P, A, B, M and W indicate the appropriate subsystems as defined in the APPROACH Section of this paper.

1) Hydrocarbons
   - .1) methane, ethane, other alkanes; G:HPABM
   - .2) ethene, other alkenes, terpenes; G:PAB, E:P
   - .3) ethyne, other alkynes; G:PM, E:HP
   - .4) benzene, alkylbenzenes, indan, naphthalene; G:M (solvents), E:HPAB

2) Nitrogen Containing
   - .1) nitrogen oxides - N_2O, NO, N_2O_3, NO_2, N_2O_4, N_2O_5, NO_3, N_2O_6; G:PAMW (fuels, atmospheric reactions), E:HPAM
   - .2) organic nitrogen oxides - RNO (nitroso), RONO (nitrite), RNO_2 (nitro), $RONO_2$ (nitrate), R_2NNO (nitrosamine); G:M (fuels), E:HPAB
   - .3) amines - NH_3, RNH_2, R_2NH, R_3N, R_4N^+; G:HPABW (cleaning and disinfecting compounds), E:HPAB
   - .4) hydrazines - N_2H_4, $RNHNH_2$, RNHNHR, R_2NNH_2, R_2NNR_2; G:M (fuels), E:HPABM
   - .5) amine oxides - NH_2OH, RNHOH, R_2NOH, R_3NO; G:M (possibly atmospheric reactions), E:HPAB
   - .6) amino alcohols - 2-aminoethanol; G:W (carbon dioxide scrubber), E:HPA
   - .7) cyanides - HCN, RCN; G:PAM (pyrolysis), E:HPAB
   - .8) cyanates (ROCN), isocyanates (RCNO); G:MW (degradation of urea and polymers), E:HPAB
   - .9) thiocyanates (RSCN); G:P, E:HPA
   - .10) nitrogen heterocycles - pyridine, indole, skatole; G:HPA(?)BW, E:HP

3) Oxygen Containing
   - .1) oxygen (in some subsystems), ozone, oxygen radicals (O, OH); G:PAM (atmospheric reactions), E:HPABM
   - .2) carbon monoxide; G:H(?)BM, E:HPAB
   - .3) water (in some subsystems); G:HPBW, E:MB
   - .4) alcohols and phenols (ROH), ethers (R_2O); G:HPBM, E:HP
   - .5) hydrogen peroxide (H_2O_2), hydroperoxides (RO_2H), peroxides (R_2O_2); G:AM (fuels, polymerizing agents), E:HPABM
   - .6) aldehydes (RCHO), ketones (R_2CO); G:HPB, E:HPB
   - .7) carboxylic acids (RCO_2H); G:HPB, E:HPBM
   - .8) carboxylic esters (RCO_2R); G:HPBM, E:HP
   - .9) oxygen heterocycles - furan, coumarin; G:PB, E:HP

4) Halogen Containing (Fluorine, Chlorine, Bromine, Iodine)
   - .1) chlorine (Cl_2), hypochlorite (ClO^-), iodine (I_2); G:M (fumigants, disinfectants), E:HPABM
   - .2) hydrogen halides (HF, HCl); G:MW, E:HPABMW
   - .3) phosgene ($COCl_2$); G:M (pyrolysis), E:HPABM
   - .4) haloalkanes (RF, RCl, RBr, RI); G:PA(?)B(?)M (macroalgae produce organic compounds of Cl, Br and I;cleaning solvents, refrigerants, polymers), E:HPABW
   - .5) fluorosilane (R_3SiF); G:M (trace contaminant in silicones), E:HPABMW

5) Silicon Containing
   .1) silanes (R_3SiH), silanols (R_3SiOH), siloxanes ($R_3SiOSiR_3$); G:M (silicones), E:P(?)W
   .2) orthosilicate esters ($[RO]_4Si$); G:M (coolant fluid), E:HPW

6) Phosphorus Containing
   .1) phosphate esters ($[RO]_3PO$); G:MW (flame retardant, plasticizer, hydraulic fluid, disinfectant), E:HPAB

7) Sulfur Containing
   .1) sulfur oxides - S_2O, SO, SO_2, SO_3; G:MW (atmospheric reactions), E:HPABM
   .2) organic sulfur oxides - sulfoxides (R_2SO), sulfones (R_2SO_2), sulfate esters (R_2SO_4); G:M, E:HPABM
   .3) sulfides - hydrogen sulfide (H_2S), thiols (RSH), thioethers (R_2S), disulfides (R_2S_2); G:PA(?)W, E:HP
   .4) carbon disulfide; G:M, E:HP
   .5) sulfur heterocycles - thiophene, thiofuran; G:B, E:HP

8) Macroorganics
   .1) pollen, bacteria, virus, spores; G:HPAB, E:HPABM
   .2) hair, skin, excreta; G:H, E:HBM
   .3) abraded material; G:M (lint, torn velcro hooks), E:HM

9) Metals
   Metals which might be generated in trace amounts by the machine subsystem and which would be biologically hazardous (depending on the chemical form and the biological species) are: Al, Sb, As, Ba, Be, Bi, B, Cr, Co, Ga, Ge, Au, In, Pb, Li, Mn, Hg, Ni, Pd, Pt, Re, Rh, Se, Ag, Ta, Sn, Ti, Zr.

TABLE 2. Airborne Trace Contaminants on Shuttle Missions

These compounds were recovered from gas sample bottles, activated charcoal filters or lithium hydroxide canisters after shuttle missions and were identified by GC/MS. They are grouped according to the classification scheme in Table 1.

1.1) methane, pentane, hexane, heptane, octane, nonane, decane, 2-methylpentane, 2,2,4-trimethylpentane, methylcyclopentane, cyclohexane, 2-methylhexane; other incompletely identified alkanes: C4-alkane, C5-alkane, C6-alkane, C7-alkane, C8-alkane, C9-alkane, C10-alkane, C11-alkane, C12-alkane, C13-alkane, C14-alkane, methylethylcyclopentane

1.2) 1-pentene, 2-methyl-1,3-butadiene, limonene; other incompletely identified alkenes: butene, pentene, C4-alkene, C7-alkene, C8-alkene, C9-alkene, C10-alkene

1.4) benzene, methylbenzene, 1,2-dimethylbenzene, 1,3-dimethylbenzene, 1,4-dimethylbenzene, butylbenzene, ethylbenzene, ethenylbenzene, propylbenzene, indan, 2-methylindan, napthalene; other incompletely identified alkylbenzenes: C3-substituted benzene, C4-substituted benzene, dimethyl ethenylbenzene

2.3) ammonia

2.7) ethanenitrile

3.2) carbon monoxide

3.4) methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol, 2-methyl-2-butanol, 1,2-ethanediol, diethoxymethane, 2-ethoxyethanol, 3-ethoxy propene

3.6) ethanal, propanal, propenal, butanal, pentanal, hexanal, heptanal, 2-ethylhexanal, 2,4-hexadienal, benzaldehyde, propanone, 2-butanone, 2-hexanone, 2-heptanone, 3-heptanone, 4-methyl-2-pentanone, 6-methyl-2-heptanone; an unidentified C7-ketone

3.7) acetic acid, 3-oxobutanoic acid

3.8) ethyl formate, ethyl acetate, propyl acetate, butyl acetate, 2-butyl acetate, 2-methylpropyl acetate, 2-ethoxyethyl acetate, ethyl propanoate, methyl 2-methylpropenoate, ethyl butanoate, diethyl o-phthalate; an unidentified C5-ester

3.9) 1,4-dioxane, furan, benzofuran, 2-methylbenzofuran

4.4) 1,1,1-trichloroethane, 1,1,2-trichloro-1,2,2-trifluoroethane, 1,1-dichloroethane, 1,1-dichloroethene, 1,1-dichlorotetrafluoroethane, 1,2-dichloroethane, 1,2-dichloropropane, bromotrifluoromethane, chloroethane, chloroethene, chlorofluoromethane, chloromethane, chlorotrifluoroethene, dichlorodifluoroethene, dichlorodifluoromethane, dichloroethane, dichloroethyne, dichlorofluoromethane, dichloromethane, tetrachloroethene, trichloroethene, trichlorofluoromethane, trichloromethane, trifluoromethane

4.5) propylfluorosilane, propyldifluorosilane

5.1) hexamethyldisiloxane, octamethyltrisiloxane, decamethyltetrasiloxane , dodecamethylpentasiloxane, tetradecamethylhexasiloxane, hexadecamethylheptasiloxane, octadecamethyloctasiloxane, eicosamethylnonasiloxane, docosamethyldecasiloxane, hexacosamethyldodecasiloxane, hexamethylcyclotrisiloxane, octamethylcyclotetrasiloxane, decamethylcyclopentasiloxane, dodecamethylcyclohexasiloxane, tetradecamethylcycloheptasiloxane, hexadecamethylcyclooctasiloxane, propylsilane, triethylsilane, trimethylsilanol

7.3) dimethylsulfide

7.4) carbon disulfide

N86-19924

OBSERVATIONS ON GAS EXCHANGE AND ELEMENT RECYCLE WITHIN A GAS-CLOSED ALGAL-MOUSE SYSTEM

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ABSTRACT

Life support systems based on bioregeneration rely on the control and manipulation of organisms. Algae are potentially useful for a variety of CELSS functions including the revitalization of atmospheres, production of food and for nitrogen fixation. We report the results of experiments conducted with a gas-closed algal-mouse system designed to investigate: 1) gas exchange phenomena under varying algal environmental conditions, and 2) the ability of algae to utilize oxidized mouse solid waste.

Inherent instabilities exist between the uptake and release of carbon dioxide (CO_2) and oxygen (O_2) by the mouse and algae in a gas-closed system. Variations in light intensity and cell density alter the photosynthetic rate of the algae and enable short-term steady-state concentrations of atmospheric CO_2 and O_2. Different nitrogen sources (urea and nitrate) result in different algal assimilatory quotients (AQ). Combinations of photosynthetic rate and AQ ratio manipulations have been examined for their potential in stabilizing atmospheric gas concentrations in the gas-closed algal-mouse system.

Element cycling experiments include wet oxidation of system waste materials for use as an algal nutrient source. Oxidized waste products demonstrate inhibitory properties although dilution has been shown to allow normal algal growth. Characterization of the nature of the inhibitory material has begun.

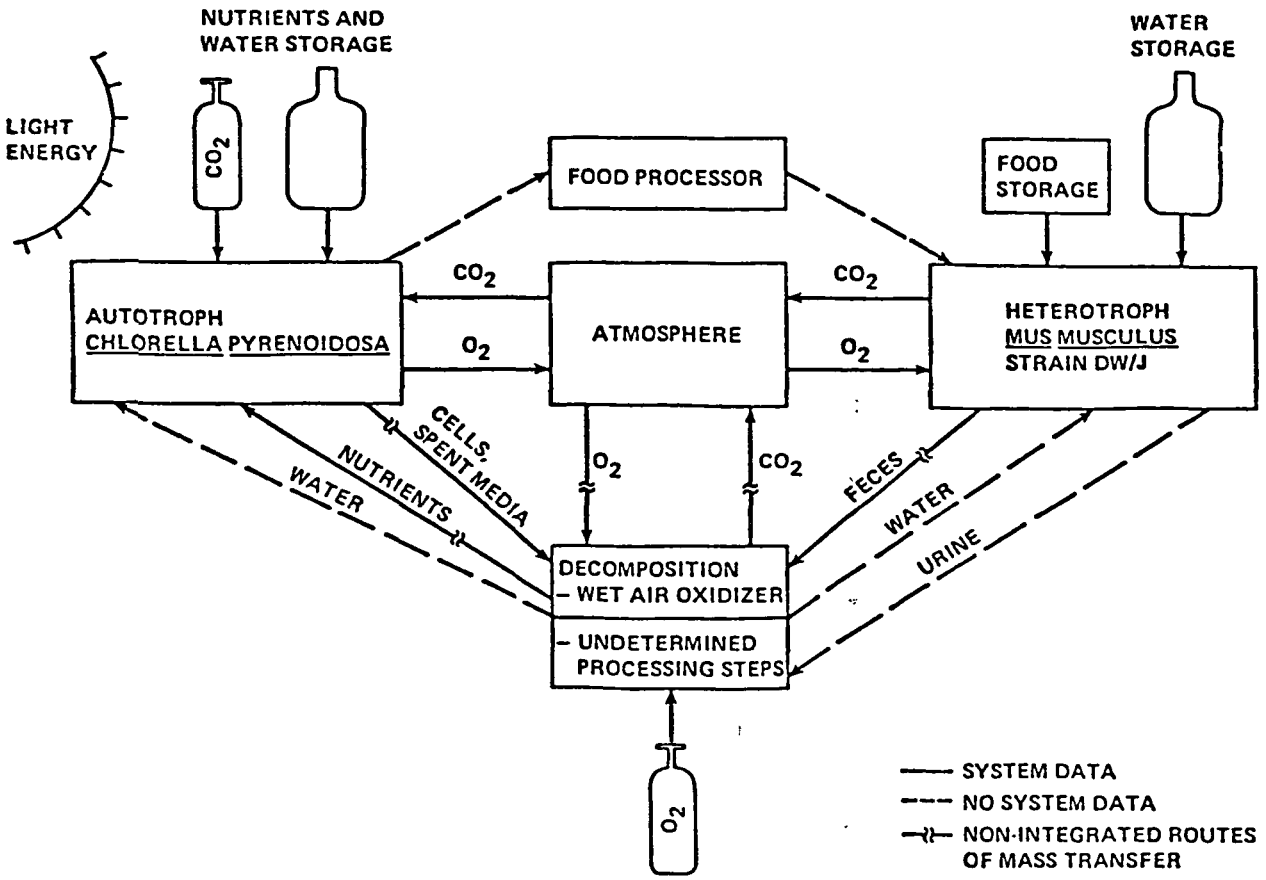

FIGURE 1. EXPERIMENTAL GAS-CLOSED MOUSE-ALGAL-WET OXIDIZER SYSTEM

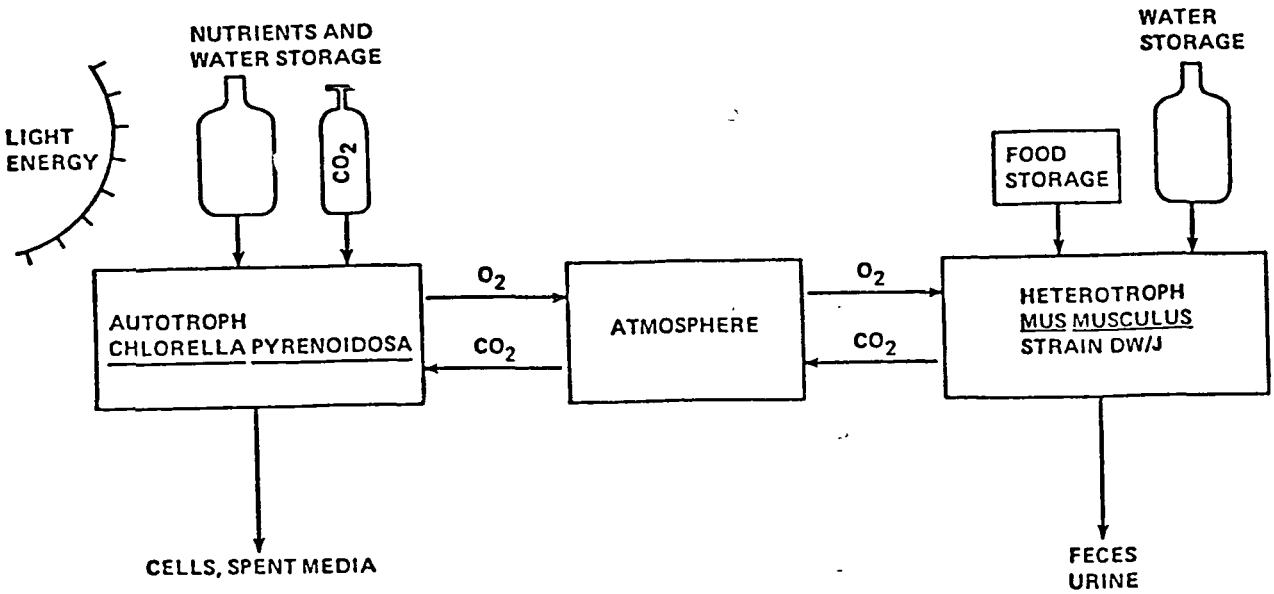

FIGURE 2. EXPERIMENTAL GAS-CLOSED MOUSE-ALGAL SYSTEM

INTRODUCTION

The development of controlled ecological life support systems (CELSS) relies, in part, on the ability to manipulate and control the organisms which are a part of the system. Algae are considered useful for several CELSS functions including the revitalization of atmospheres, production of food and for nitrogen fixation. Techniques to accomplish these functions using algae enhance the development of an operational CELSS by addressing control issues, and enhancing reliability and stability by increasing the available options.

The research reported is directed at understanding how an integrated CELSS might function and particularly to identify any problems which may occur. The strategy has been to control a small model CELSS based on algae and mice and to study its behavior under different operating conditions. The major system components (Fig. 1) are; the green alga Chlorella pyrenoidosa, the dwarf mouse strain DW/J and a bench scale wet-air oxidation (WAO) reactor. Primarily we are concerned with the atmosphere behavior within the gas-closed algal-mouse system, additionally we have initiated studies to determine the degree of element recycle possible. As indicated in Figure 1 the waste processing subsystem is not physically coupled to the algal-mouse system. This limits the ability to determine the recyclability of the system although preliminary analysis of system interactions is possible.

METHODS

Figure 2 schematically represents the operation of the algal-mouse system without the incorporation of waste oxidation into the system. The majority of data presented in this paper is concerned with the dynamics of gas exchange between the algae and the mouse under a variety of algal growth conditions. Parameters varied are the optical density of the cultures, the light intensity which the algal reactors receive and the nitrogen source in the algal media. Carbon dioxide is supplied either from cylinders or from the mouse reactor. Mouse food and water are externally supplied and oxygen is supplied from cylinders or the algal reactors.

Figure 3 shows details of the experimental algal-mouse system. It is comprised of three gas-tight reactors, two of which support continuous algal growth and one which houses a mouse. The system is designed to operate in either a gas flow-through mode or in a gas-closed mode. Measurement of atmospheric concentrations of oxygen (O_2 and carbon dioxide (CO_2) is accomplished with paramagnetic and infrared analyzers, respectively. The algal reactors are operated as turbidostats with optical density of the cultures being controlled by the addition of fresh media. The operation of the system includes the use of a computer to collect data and to operate pumps and valves used to alter system states.

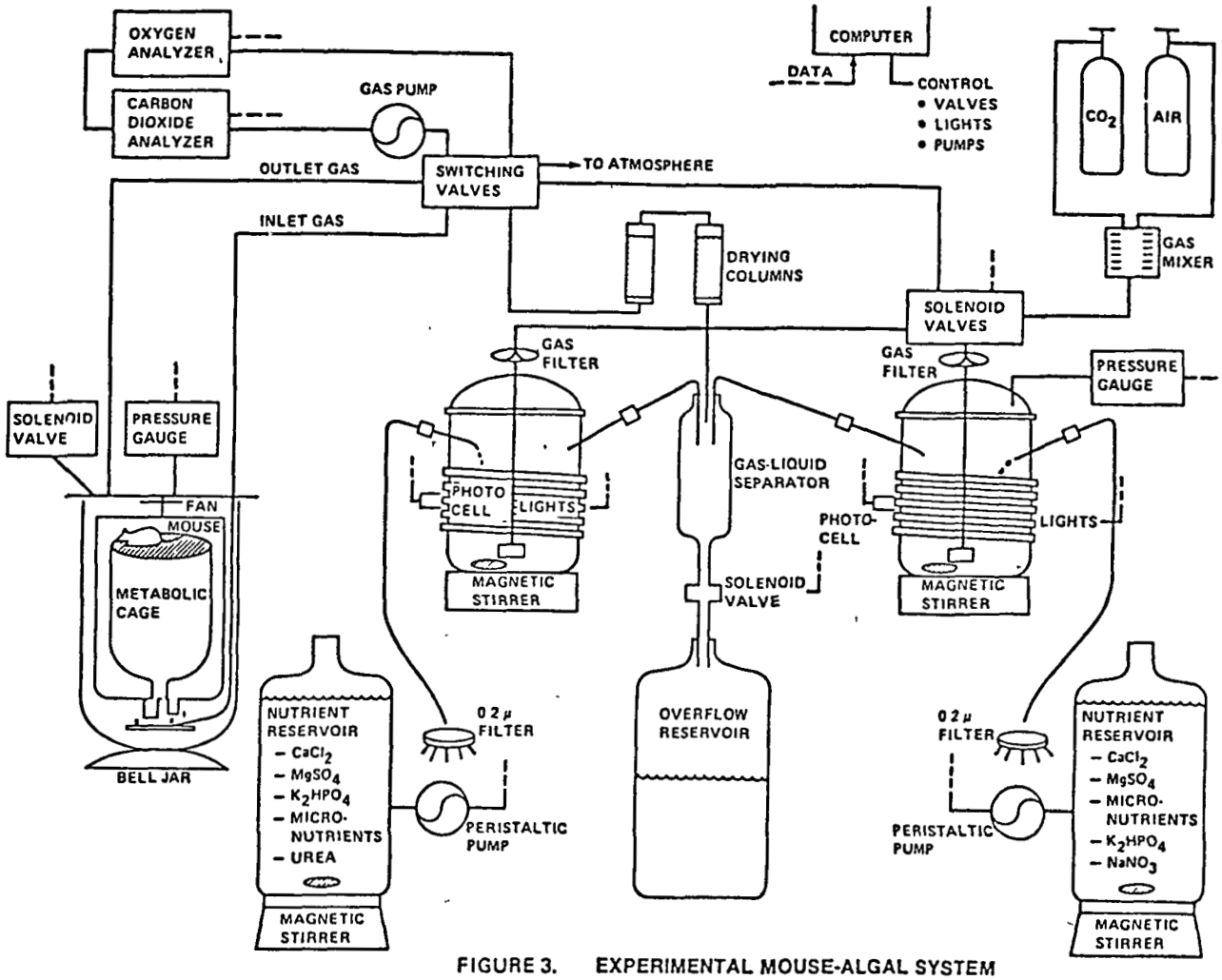

FIGURE 3. EXPERIMENTAL MOUSE-ALGAL SYSTEM

RESULTS AND DISCUSSION

Three types of gas exchange experiments are conducted using the system. Measurement of mouse respiratory quotients (RQ) is done by closing the mouse in the reactor with ambient atmospheric concentrations of CO_2 and O_2 and observing the changes in each gas concentration over time. The RQ is calculated from the change in CO_2 divided by the change in O_2 (RQ = moles CO_2 produced/moles O_2 consumed). Figure 4 graphically represents system behavior with a mouse only. Short-term measurements of mouse RQ have been determined to be 0.975 $\pm$0.06 (n=5).

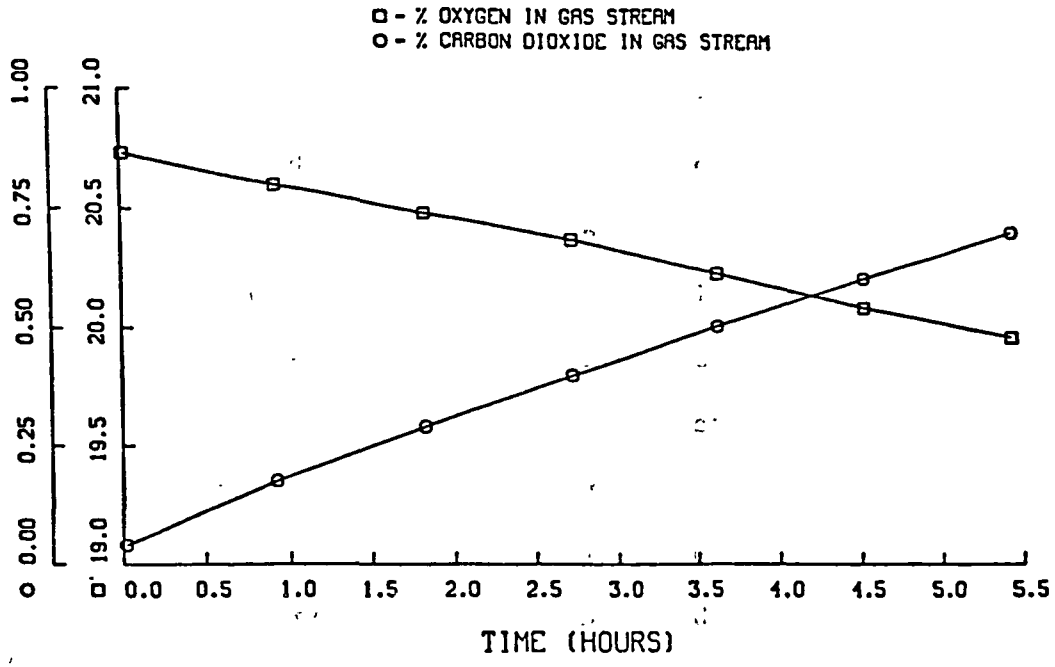

FIGURE 4. CO2-O2 RELATIONSHIP IN A GAS-CLOSED MOUSE SYSTEM
Measurement of Mouse Respiratory Quotients

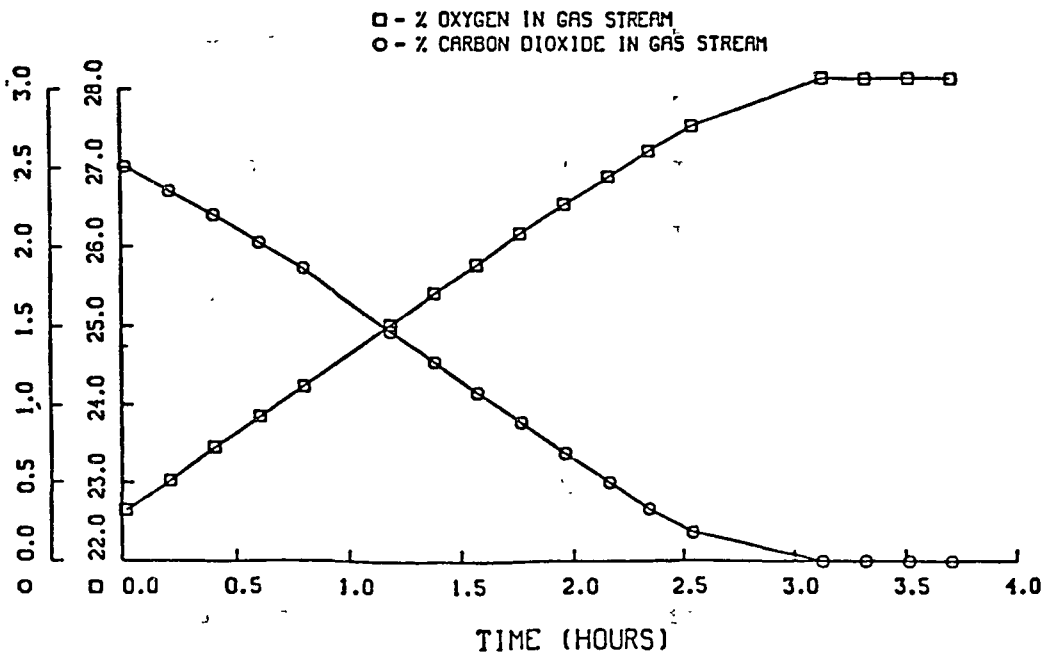

FIGURE 5. CO2-O2 RELATIONSHIP IN A GAS-CLOSED ALGAL SYSTEM
Measurement of Algal Assimilatory Quotients, Nitrate (OD 0.61)

The second type of experiments determine the algal assimilatory quotient (AQ) in a similar fashion. The algal cultures are normally supplied with CO_2 (2%) enriched air. When AQ measurements are made, the gas flow from the cylinders is stopped and the gas within the system is recirculated using a pump. The slopes of the CO_2 and O_2 concentrations are then used to calculate the AQ (AQ = moles CO_2 consumed/moles O_2 produced). Figure 5 exhibits the opposite set of responses from Figure 4, as would be expected. The third set of experiments, to be discussed later, are conducted with the mouse and algal reactors coupled.

Due to metabolic differences between the mouse and the algae the AQ and the RQ are not the same. This inherent mismatch will result in depletion of one atmospheric component. Therefore, in order to maintain stable concentrations of CO_2 and O_2 within a coupled mouse-algal system, it is necessary to control either the AQ or the RQ. Control of the RQ is possible but not a realistic option for use in a CELSS. Therefore, techniques to match the AQ of the algae to the RQ of the mouse are examined. One technique studied takes advantage of the observed difference in AQ between cultures grown on nitrate and those grown on urea. Table 1 exhibits the variation in AQ between nitrate and urea grown cultures.

TABLE 1: AQ as a Function of Nitrogen Source

	NITRATE	UREA
ASSIMILATORY QUOTIENT (AQ)	0.50 $\pm$ 0.07	0.77 $\pm$ 0.12
Sample size	(n=25)	(n=28)

The data in Table 1 was obtained from bacterially contaminated cultures. Our experience indicates that it is extremely difficult to maintain axenic algal cultures for long time periods. Therefore, it is valuable to establish AQ values for pure and contaminated cultures grown on nitrate or urea. Figure 6 exhibits the variation in AQ for pure and bacterially contaminated cultures grown on nitrate. The two uncontaminated points with AQ's near 0.45 were from a run which initially appeared to be pure, but which later showed bacterial contamination. From this information we can make the assumption that even low levels of bacterial contamination will have an effect on the apparent AQ of the algal culture. The large variation in AQ for contaminated runs is probably due to different bacterial population sizes. The uncontaminated points clustered around an AQ of 0.7 showed no bacterial

contamination, further supporting this hypothesis. Further data must be collected concerning the relative population sizes of bacteria and algae and how this affects the apparent AQ. Precise information on this interaction will allow greater control of algal gas revitalization systems.

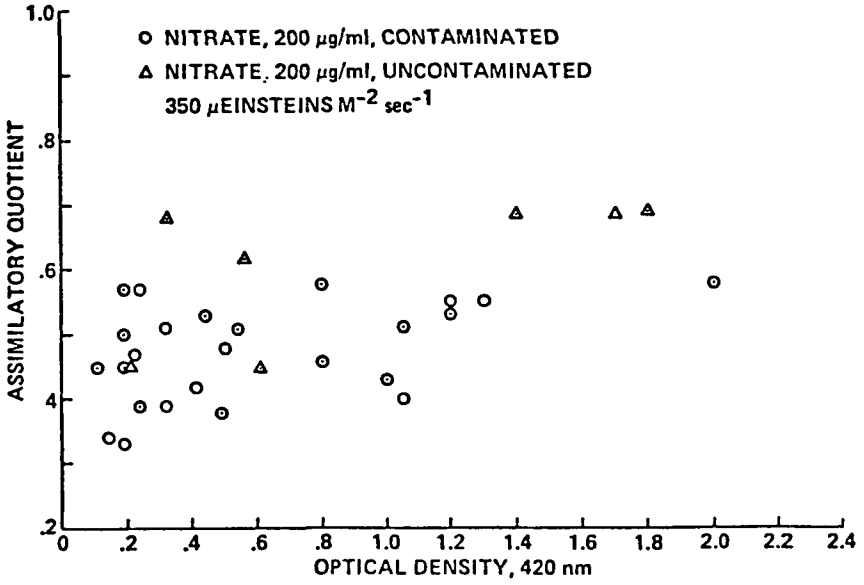

FIGURE 6. VARIATION OF ASSIMILATORY QUOTIENT AS A FUNCTION OF OPTICAL DENSITY

Another parameter which affects algal gas revitalization characteristics is the light intensity which the cultures receive. In combined algal-mouse runs variation in light intensity has been shown to allow control of gas exchange mismatches. Figure 7 demonstrates an initial system state which we refer to as a photosynthetic mode. In other words, the oxygen

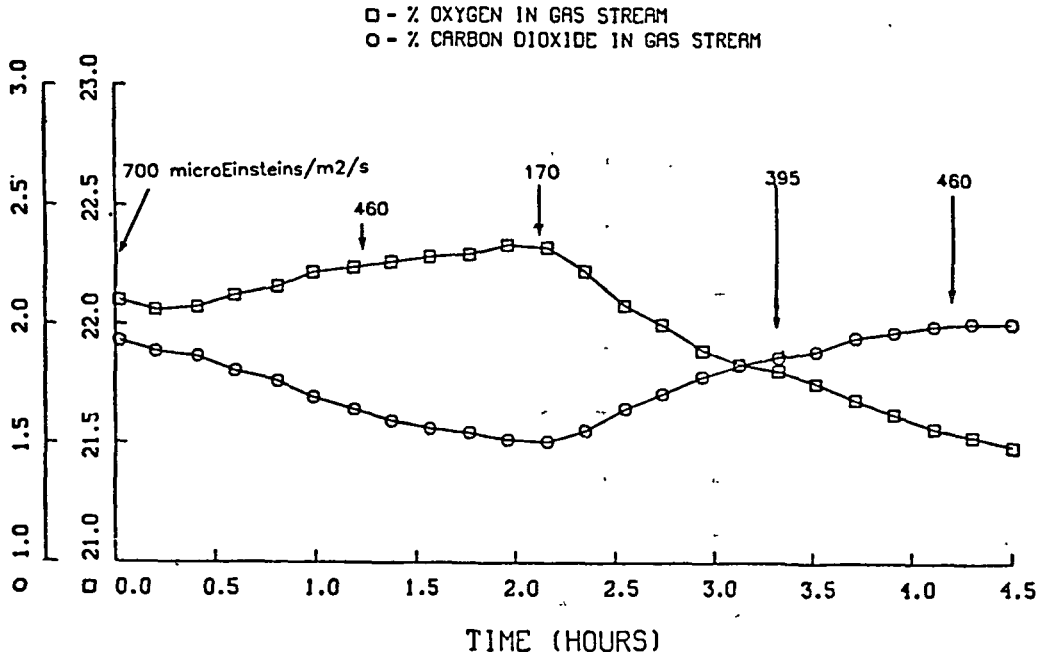

FIGURE 7. CO2-O2 RELATIONSHIP IN A GAS-CLOSED ALGAL-MOUSE SYSTEM
Effect of Variation in Light Intensity (Nitrate R1 OD 2.1 R2 OD 1.7)

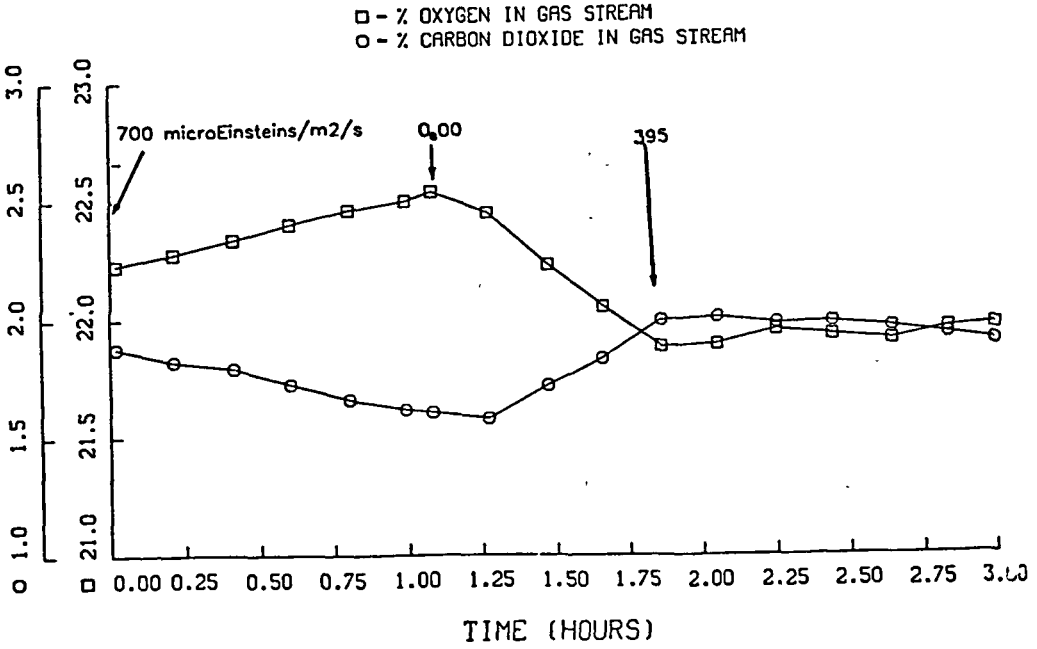

FIGURE 8. CO2-O2 RELATIONSHIP IN A GAS-CLOSED ALGAL-MOUSE SYSTEM
Effect of Variation in Light Intensity (Urea R1 OD 0.77, R2 OD 0.81)

production of the algae exceeds the oxygen uptake of the mouse. By lowering the light intensity (at 1.1 hours) we reduce the photosynthetic rate of the culture and lower the oxygen output. Further reductions in light intensity at 2.0 hours shifts the system state to a respiratory mode (i.e. oxygen consumption of the mouse exceeds oxygen production by the algae). Further changes in light intensity at 3.0 and 4.0 hours restore the system to a photosynthetic mode. Although figure 7 does not demonstrate a gas-stable system it does show that control can be achieved by photosynthetic rate manipulations. Figure 8 shows a system state in which variation of light intensity results in a relatively stable system state.

Figures 9 and 10 show what we refer to as the crossover area between photosynthetic and respiratory modes. The shaded area indicates the uncertainty associated with the data. The difference in algal AQ between nitrate and urea grown cultures is also evident in the crossover area curves. Refinement of this data will allow predictions to be made concerning the system states for cultures maintained at selected optical densities, light intensities and nitrogen sources. The significantly different gas-exchange characteristics at selected operating regimes may then be exploited for control purposes. Multiple reactors, each with distinctive gas exchange characteristics may be used to operate the system in a stable fashion with minimal control requirements.

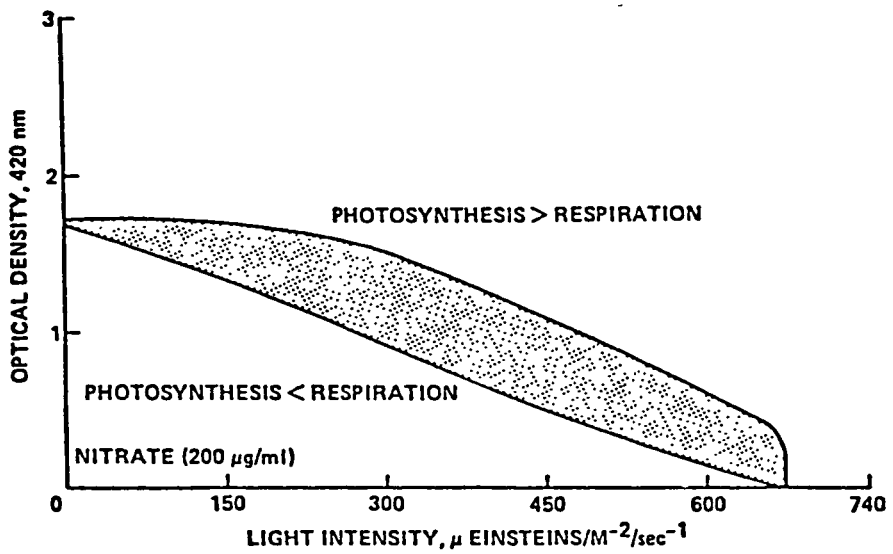

FIGURE 9. CROSSOVER AREA AS A FUNCTION OF OPTICAL DENSITY AND LIGHT INTENSITY

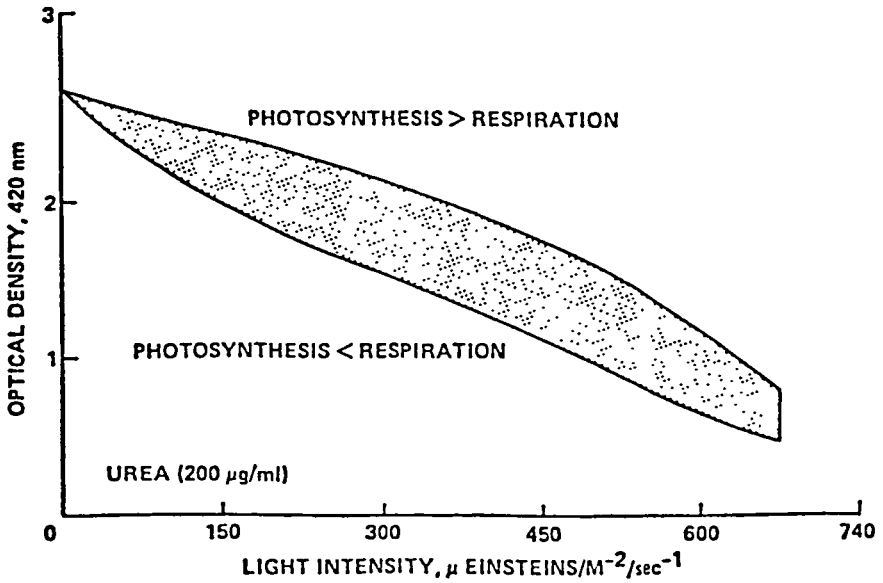

FIGURE 10. CROSSOVER AREA AS A FUNCTION OF OPTICAL DENSITY AND LIGHT INTENSITY

Figure 11 exhibits a system state in which gas concentrations show only slight variation. The operating characteristics involve only the manipulation of algal AQ by use of different nitrogen sources. The graph exhibits that two reactors, one growing on nitrate and the other on urea, can maintain stable atmospheric CO_2 and O_2 concentrations within a closed system. In order to show the feasibility of this technique for CELSS application it must be demonstrated that stability can be achieved for time periods much greater than 7 hours.

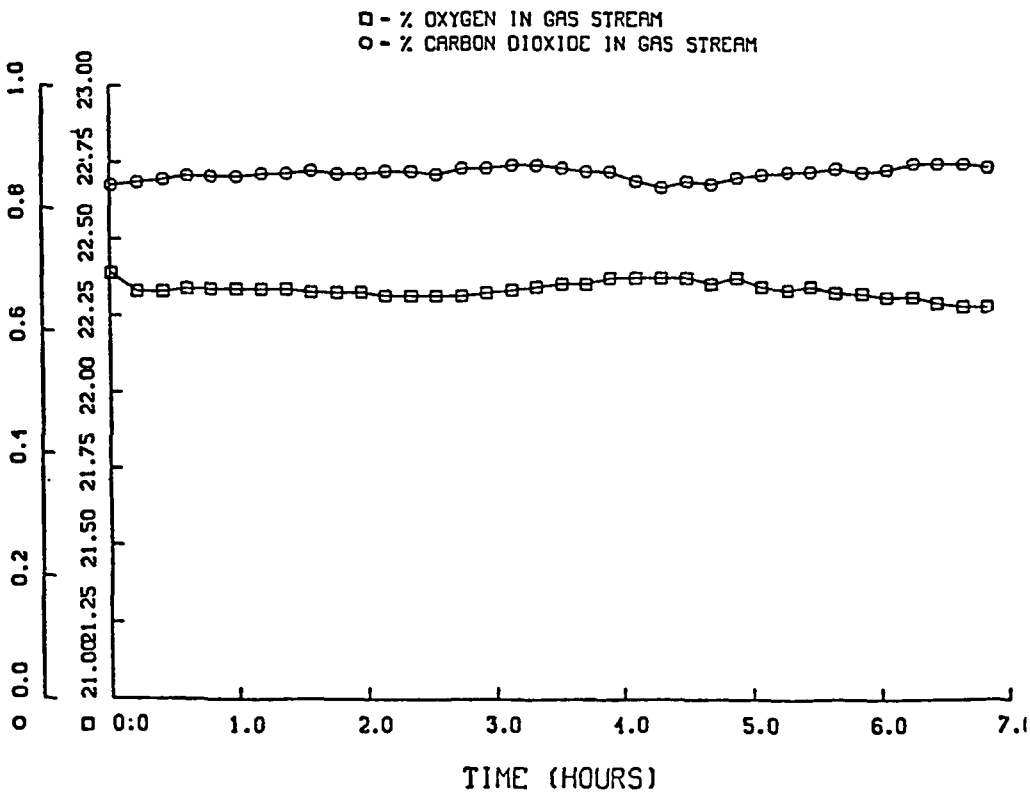

FIGURE 11. CO2-O2 RELATIONSHIP IN A GAS-CLOSED ALGAL-MOUSE SYSTEM
R1 Nitrate (OD 1.64): R2 Urea (OD 4.00): 700 microEinsteins/m2/s

The response of the system seen in Figure 11 does not agree with the response which would be predicted from the crossover area curves. The experimental conditions, as observed on the crossover area curves, would be predicted to lead to a system state in photosynthetic excess. However, a balance between photosynthesis and respiration is observed. In fact, at about 5.0 hours a respiratory trend is beginning to emerge which is directly in opposition to crossover area predictions.

There are several possible explanations for this discrepancy. First is the uncertainty of the crossover area curves. More data is required in order to refine the crossover area into a crossover point, this will allow more accurate prediction of system behavior. Secondly, the sum of subsystem behaviors (e.g nitrate and urea grown cultures) may not equal the sum of the overall system behavior. The crossover area curves were derived from urea and nitrate grown cultures operating independently, the crossover area for urea and nitrate cultures operating together may be different. To verify this hypothesis further data acquisition is required. A third possible answer may be due to the large mouse chamber volume which will significantly delay system response. Experiments conducted with a small volume mouse chamber show greater instability for combined urea-nitrate conditions, indicating that system volume is affecting observed system states. Comparison of small reactor to large reactor data and longer runs will be required in order to determine the

effect of large mouse reactor volume on system behavior.

Returning to Figure 1, we will now look at material recycling within the experimental system. As stated earlier, the system is not fully integrated which limits the ability to determine mass balance for the system. However, we have conducted a series of experiments to determine if algal growth can be supported by the fecal output of the mouse. Figure 12 shows the growth of algae on a wet-oxidized sample of mouse feces. A 1:1 dilution of the fecal wet-oxidate inhibits algal growth while a 1:15 dilution of the same material shows growth equal to a positive control.

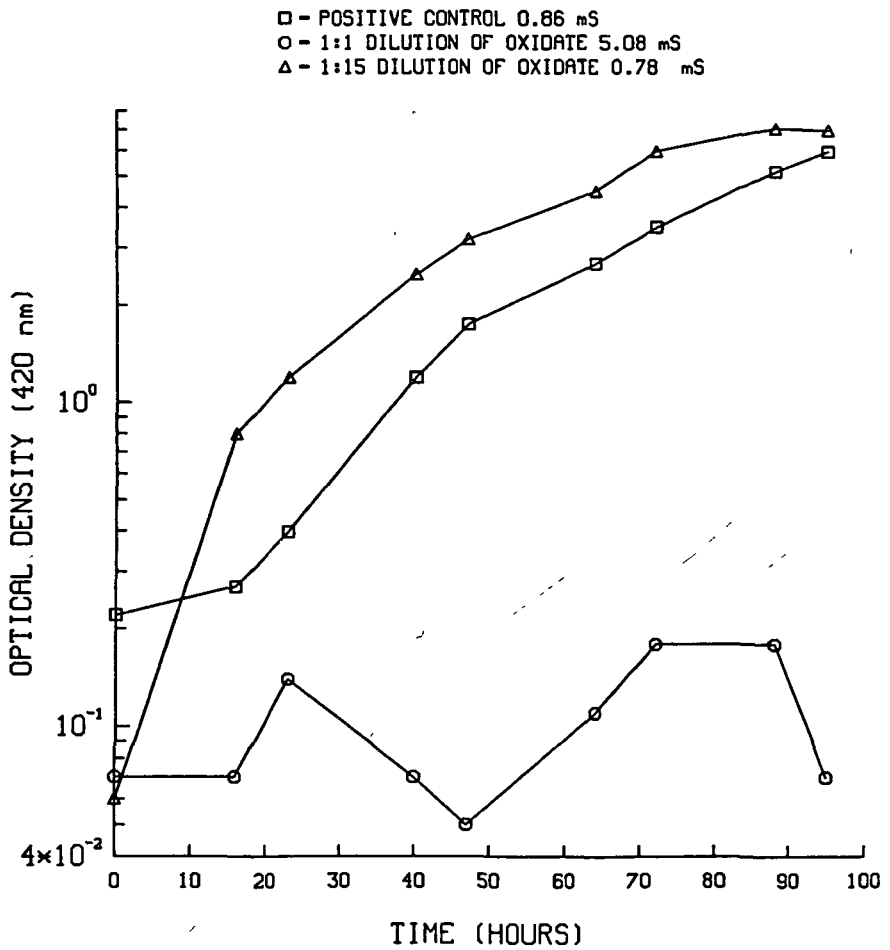

FIGURE 12. GROWTH OF ALGAE ON WET-OXIDIZED CHLORELLA

It was observed that the osmolarity of the 1:1 material was much greater than that of the normal control media. To test the hypothesis that the inhibitory effect was due to high salt concentration we ran an experiment with several different osmolarities of normal media. We found that the normal media with an osmolarity equal to the 1:1 fecal wet-oxidate showed normal algal growth. Therefore, it is unlikely that the inhibition observed was due to a high salt concentration effect. Another experiment tested wet-oxidized distilled water as an algal nutrient source. No inhibition of algal growth was observed, indicating that the wet oxidation reactor is not contributing an inhibitory substance. Therefore, we conclude that the feces is the source of the inhibition although at this time we have not determined what the inhibitory material is.

CONCLUSIONS

The use of algae in a CELSS will depend on many factors, including the ability to monitor and control the algal cultures. Work conducted under this research program has indicated that algae may be useful in the regeneration of the atmosphere within a closed spacecraft ecology. By taking advantage of the inherent characteristics of algae, endogenous control strategies have been developed which limit the amount of exogenous control energy which must be exerted on them. Production of large amounts of biomass, coupled with appropriate

processing techniques, fixation of nitrogen and minimal maintenance and control energy requirements make algae an attractive biological component for inclusion in a CELSS.

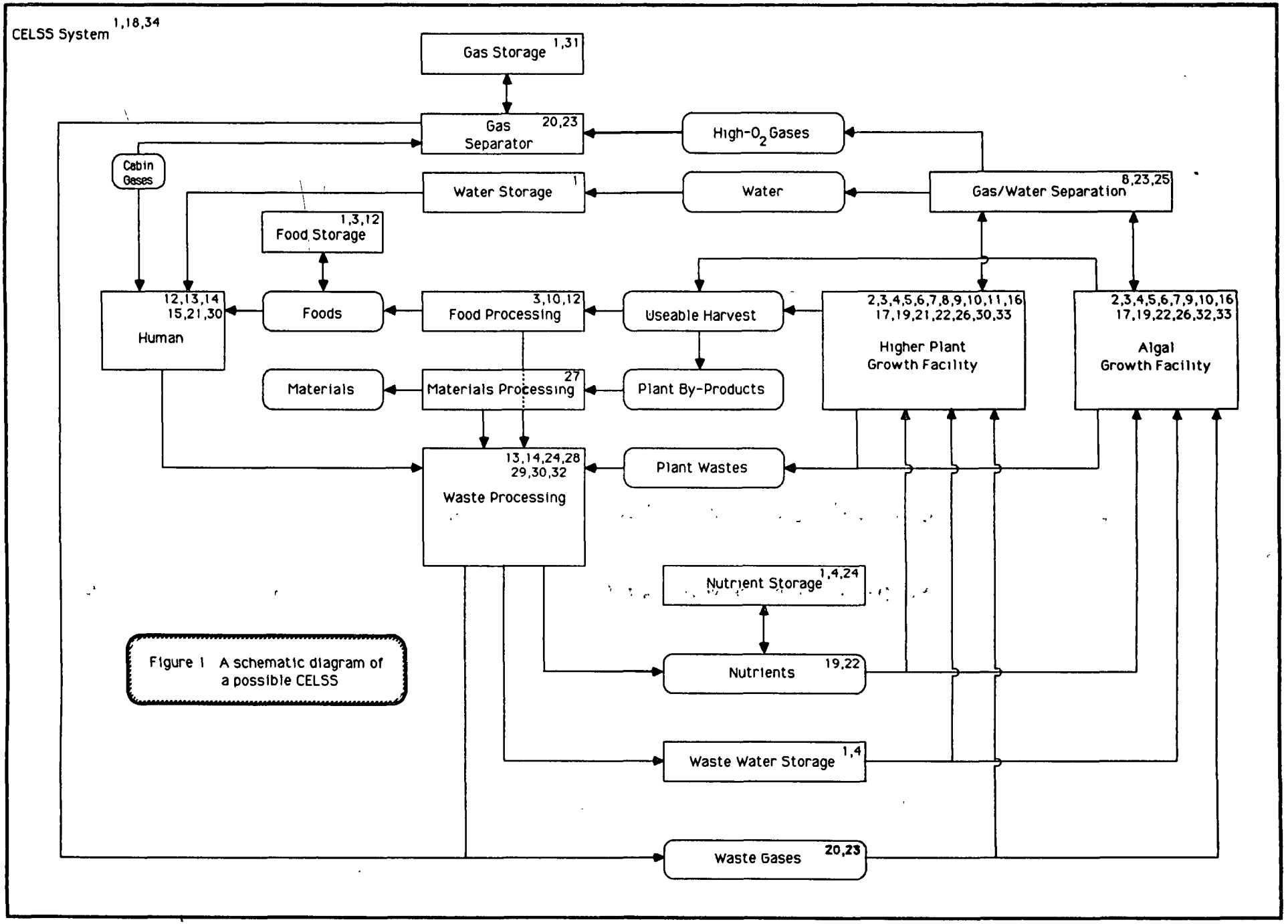

Figure 1 A schematic diagram of a possible CELSS

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CELSS SCIENCE NEEDS

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Introduction

In the development of a Controlled Ecological Life Support System (CELSS) it is periodically useful to review the project to identify those areas that are most in need of research. This paper is my own view of what is necessary to accomplish in CELSS science. It is not intended to be definitive, but to be an heuristic tool to focus attention on outstanding questions. Any list of this sort is sure to have omissions, and mine is no exception, though it is reasonable to expect that a list of science questions will continue to evolve as some of those questions are answered and while new areas of interest are being uncovered.

Despite the evident difficulties, I propose the following list of areas where further investigation is needed before we can build a workable CELSS. To make these suggestions more concrete, each of them is numbered to correspond to specific portions of the CELSS that is diagrammed in Figure 1. Each of the numbered areas will be affected by the research in question. In the spirit of this paper the details of Figure 1 are not meant to represent the final design of a CELSS. In fact, the schematic shown does not even reflect my own current thinking on that subject. Nevertheless, I hope it will be a useful "straw man" for the purpose intended.

CELSS Science Needs

Biological Sciences

Ecology

1. Investigate the dynamical behavior of the system, and adjust the design to minimize buffer sizes, while assuring system stability and safe operation.

2. Develop and characterize a benign microbial community to enhance plant growth, and to provide protection against plant pathogens.

Genetics

3. Provide novel cultivars that will increase yields within a small volume. Select plants that will be able to thrive at less than one gravity.

Plant Pathology

4. Develop prevention and control measures for potential plant pathogens.

Plant Physiology

5. Characterize optimal growth needs of crop plants useful for a CELSS.

6. Develop control methods for crop plants (control growth, transpiration, etc.).

7. Characterize the gas exchange capacity of crop plants under different growth regimes, and during different phases of the plant's life cycle.

8. Explore the use of transpiration water as drinking water under the full range of growth regimes envisioned.

9. Characterize the production of secondary compounds by crop plants under the growth regimes envisioned.

10. Evaluate the potential for mixed-crop and continuous-harvest systems under CELSS constraints.

11. Develop a method to orient crop species in micro-G.

Medical Sciences

Nutrition Science

12. Fully define human nutritional requirements, and determine the extent that they can be met by a CELSS.

13. Characterize the waste materials produced by humans on a CELSS-compatible diet.

Human Physiology/Space Physiology

14. Characterize human metabolic activity and gas physiology in the micro-G environment over a long-duration mission.

Toxicology

15. Characterize the long-term effects of exposure to low levels of plant secondary compounds.

Computer Science

Artificial Intelligence/Robotics

16. Develop the capability for remote monitoring and control of plants and algae.

17. Develop the capability for automated harvesting, with quality assurance and food preservation needs being met.

C - 4

Cybernetics

18. Develop methods for overall system control and failure analysis, perhaps by integrating a computational model of the system with the system itself.

Chemistry

Analytical Chemistry

19. Develop monitoring capabilities for the nutrient solutions and waste streams.

20. Develop monitoring capabilities for mixed-gas streams.

21. Develop capabilities to monitor small quantities of plant secondary compounds and potential toxins in real time.

Physical Chemistry

22. Develop appropriate models to predict the behavior of nutrients in solution.

23. Develop appropriate models to predict the behavior of mixed-gas streams.

24. Develop a method for separation and storage of nutrients from the waste stream.

25. Optimize gas-separation methods to match CELSS power and volume constraints.

26. Develop methods for control of nutrient solutions in the growth chambers.

Materials Chemistry

27. Develop processes for the small-scale handling of plant by-products.

Waste Chemistry

28. Perfect techniques for physical-chemical waste treatment.

29. Investigate hybrid waste-treatment techniques (to include the use of genetically engineered microbes to detoxify waste).

30. Develop specific techniques to remove low-level toxins and plant secondary compounds from both liquids and gases.

Physics

Fluid Dynamics

31. Develop systems for mixed-gas storage in micro-G.

32. Develop methods for mixing the algal-growth media and controlling the waste-processing streams in micro-G.

Optics

33. Develop an optimized system to provide photosynthetically active light to the growth chambers.

Solid-State/Atomic Physics

34. Determine the most efficient way to provide the radiation shielding necessary to protect the system from long-term damage and performance degradation.

MICROBIAL SYSTEMS

Dr. Lester Packer, Chairman

N86-19926

DESIGN CONCEPTS FOR BIOREACTORS IN SPACE

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INTRODUCTION

Bioprocessing in space and in extra-terrestrial facilities is both a logical extension of bioprocessing needs on earth and in some cases a better alternative to obtain biomass and biologicals rapidly and efficiently. In the context of CELSS, trade-off studies on food regeneration strategies may point to microbial food production as the choice in terms of efficiency, economy and fault-tolerance. Even otherwise, their value as redundant and supplementary food sources cannot be overemphasized. Work needs to be done to identify and even genetically modify microbial strains to provide an optimum(70:20:10)[1] mixture of carbohydrates, proteins, and lipids. The promise of high energy efficiencies and weight/volume ratios does provide a tremendous incentive to undertake such work expediently and systematically. While this work is progressing, parallel effort must be undertaken to address the problems of operating bioprocessing units in microgravity.

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Microgravity is not expected to have any significant effect on basic biokinetic rate of biological reactions. However, the associated operations of mixing nutrients/reactants and separating products will greatly depend upon the magnitude of gravitation. These two, in turn, will affect significantly the production rate of bioprocessing units. Any bioprocessing unit may contain one or more bioreactors. The mixing/separation may be external to the bioreactor or integral with the operation of bioreactor itself. Two and three phase mixing/separation operations are too complex to model from first principles. There are no simple correlational procedures to convert engineering data and tools used for terrestrial designs for applicability to microgravity. One can choose between two approaches to arriving at a successful design of a bioreactor for use in microgravity: (1) Build rightaway a candidate bioreactor and associated instrumentation based on terrestrial experience and test it in microgravity. Conduct subsequent tests to modify the hardware and operating conditions/procedure to optimize the design. (2) Conduct an experimental program for obtaining key engineering data under conditions of microgravity and use this data base to develop design tools and procedures for the design of space bioreactor systems for a broad range of applications. The authors tend to prefer the second approach

as the one providing the most value for the money.

BACKGROUND

A major research thrust of NASA's CELSS program is to develop practical and energy-efficient ways to recycle all of the materials involved in life processes so that a controlled closed life support system requires as little resupply and energy input as possible to sustain spacecraft crews for long term space habitation.

1. Microorganisms as Food Sources

Conventional food sources consist of higher plants and animals. Unconventional food sources for human consumption are photosynthetic algae and bacteria and non-photosynthetic bacteria, yeasts and fungi. Conventional food sources are highly palatable, but require long lead times to produce. Under conditions of epidemic loss of conventional food sources, recovery may be prolonged or impossible. The photosynthetic energy efficiency of higher plants is less than 3%. Even though conventional food sources will be our best choice on account of our excellent culinary experience with them, they are not the most abundant and dependable in the context of an enclosed extra-terrestrial habitat.

Survival under conditions of 'drought' during a long term space mission can be realistically estimated to be near zero. On the contrary, microbial food sources such as algae, yeast and fungi are unconventional and are usually used only as supplements to conventional food. Their nutritional content based on current data can be presumed to be adequate to meet human dietary needs. Microbial food production systems have the advantages of lower weight/volume requirements[2] over conventional plant/animal production systems and they also account for superior energy utilization in the production of carbohydrates from CO_2 and H_2O. A quick summary of what it takes to produce an acceptable menu of food items for space habitats can be found in the literature[2] and the problems do not seem to be intractable. Therefore it is not a far-fetched assumption that adequate nutritional composition can be achieved using microbial food sources and the microbial mass can be made palatable to humans through development of suitable food processing techniques.

For the common food microorganisms, evaluations of nutritional adequacy have been made and the methodologies are well known[3]. However, for methylotrophic organisms and other heterotrophs, nutritional adequacy evaluations are limited in their scope and depth. Work at the Jet Propulsion Laboratory has focussed on the use of a candidate

methylotrophic yeast, Hansenula Polymorpha. Genetically modified strains of this microorganism were selected for a high glycogen phenotype[4]. Since this organism was refractory to biochemical analysis due to a very stable cell wall, new analytical approaches had to be developed and tested. These approaches have led to methods which can be used to accurately characterize and evaluate even those yeast strains with difficult lytic characteristics for nutritional adequacy in a CELSS context. In addition, nutritionally augmented strains of the methylotrophic yeast, H.Polymorpha have been produced that contain almost twice as much edible carbohydrate as the wild type strain[5]. Such techniques are especially valuable since most of the microorganisms have low levels of carbohydrate in terms of human nutritional needs.

2. Microbial Growth Chambers

There have been efforts made by both U.S. and Soviet scientists to design microbial growth chambers. Two plans have been suggested by Martin-Marietta Corporation[6,7]. One involves a flow circulation loop and the other is a cylindrical fermenter design. The flow circulation model is designed for both production and collection of cell mass, however it is more suitable for bioprocessing than cell harvesting. The cylindrical fermenter is more like the

standard terrestrial fermenter adapted to accommodate microgravity environments. In the early days of CELSS, the use of hydrogen bacteria as regenerative food was considered[8]. An apparatus for operating such a system was suggested by scientists working for NASA. The Soviets too did some preliminary work on H_2 bacteria growth in their "flying oasis" which was reported to have flown on Soyuz 13 in 1973[9]. Both NASA and Soviet flight programs included algal growth chambers which were tested in various stages of development from ground based studies to flight models[10,11,12,13]. Neither program generated sufficient data to evaluate their progress.

For over a decade, NASA personnel at Johnson Space Center have directed the development of a bioprocessing system that includes both the production of pharmaceutical products and their separation in space. The bioseparation process has been demonstrated on successive STS missions over the past two years. Work in the bioproduction area has not progressed quite as rapidly due to the concentrated effort on bioseparation. The project is designed to bring about the culture of mammalian cells to produce pharmaceutical products. Terrestrially, the culture of cells is compromised by sedimentation and oxygen transfer limitations. Microgravity can help overcome these problems. Over the past

few years, U.S. and European flight experiments have shown positive microgravity effects on eucaryotic cell growth and cell size. A space bioreactor for cell culture has been proposed for operation in microgravity[14]. It is designed for eventual tandem operation with continuous flow electrophoresis. The elimination of sedimentation or bubble buoyancy is thought to aid in the growth and maintenance of mammalian cells which are extremely shear-sensitive. The purpose of these attempts is to enhance the production of pharmacologically important hormones and other medical products. It is important to note that design and performance data from the mammalian cell growth programs are not immediately applicable to the growth of microorganisms. The requirements for microbial food production units in a CELSS environment include high intensity cultures requiring significantly higher quantities of oxygen and mixing rates which would probably shear mammalian cells.

SPACE BIOREACTOR - DESIGN CONSIDERATIONS

Since the emphasis in this paper is on unconventional food production for CELSS, the following discussion will be concerned with fermenters as microbial growth chambers. Bioreactors for other applications will have many characteristics in common with fermenters and their design

and operation can have many similarities to the design and operation of fermenters.

A typical industrial fermenter on earth will not operate in microgravity for the following reasons:

(1) Gas bubbles will not rise through the fermentation media due absence of significant buoyancy forces.
(2) There will not be a single level separating the gas and the liquid and gas bubbles may not disengage at these multiple interfaces.
(3) As gas transfer efficiencies of 100% are practically unachievable on earth even in the absence of other fermenter constraints, there is no reason to believe that they will be attainable in microgravity. Phase separations are not spontaneous.
(4) Foaming is likely to be an even more severe problem as surface forces causing foaming will be more predominant in microgravity.
(5) The fermenter may oscillate between continuous liquid phase and continuous gas phase or both may coexist in various regions of the fermenter volume.

In submerged culture, aerobic microorganisms grow very rapidly until at a critical cell mass they are consuming all

the oxygen that a fermenter can supply. For growth conditions of relevance to microbial food production, the biological kinetics are sufficiently fast that the rate of transfer of oxygen and rate of removal of carbon dioxide determine the microbial growth rate. Surface area of gas bubbles and internal convection within air bubbles control the rate at which oxygen transfers to the growing cells. Bubble size as produced at a sparger orifice is controlled by the balance of gravitational forces and surface tension. In microgravity, this implies that the bubble size will increase rapidly, providing much less interfacial area per unit volume of the fermenter severely limiting gas transfer. Bouyancy forces also act to enhance gas transfer by participating in more intense surface renewal and gross mixing. But in microgravity these forces are too small to be significant. Therefore, to provide efficient mixing of gas and liquid inside the fermenter, stable colloidal gaseous dispersions must be generated within the fermenter with the help of suitable surfactants[15]. Even though the surfactants will inhibit the mass transer rate across the gas liquid interface, through proper choice of concentration of surfactants it is possible to ensure that the increase in interfacial area more than compensates for the inhibitory role of surfactants. A second approach to overcome the problems of bubble size and lack of bouyancy is not to

generate bubbles at all in the liquid medium but to transfer the gases across a suitable membrane at a rate equal to the dissolution rate gases in the liquid. A third approach will be to induce centrifugal body forces in the liquid medium and provide for bouyancy forces for gas bubbles to "rise" to the center. As in terrestrial designs, baffles and other flow redirections can be provided inside the fermenter to augment the mixing intensity.

In microgravity reluctance for dissimilar phases to separate is a serious design issue. If colloidal gas bubble suspensions are employed, at the end of required cell growth, the surfactants must be disabled without toxicity and detriment to nutritional quality. Separation of gases from liquid and separation of cell mass from liquid will necessitate suitable membrane transfer or centrifugal separation units.

The problems associated with mass transfer can also be expected with heat transfer. Natural convection will be too feeble in microgravity and forced circulation over heating or cooling surfaces will be necessary which is also the preferred approach in terrestrial designs as well.

The problems of mixing and separation in microgravity will

have to be overcome in suitable ways depending on the type of fermenter operation. Fermenters can be operated in batch mode, fed-batch mode, and continuous mode. In the continuous mode, the choice is between a single plug flow design and a multiple CSTR design with cocurrent or countercurrent gas flow.

Fermenter configurations vary owing to emphasis on one or more of the following key parameters: Oxygen transfer intensity, Power economy, Cell growth rate, Production rate of other products.

NEED FOR ENGINEERING DATA

In addition to physical configuration of a space fermenter, a designer must determine quantitatively the following:

(1) Power required for mixing.
(2) The space-velocity or space-time which are measures of fermenter volume or the amount of time the nutrients and gases must remain in the fermenter for the required production rate of cell mass.
(3) The mass transfer rate achievable and the associated gas bubble size, mixing intensity and interfacial area.
(4) The heat transfer rate necessary to maintain the

temperature within the optimum range.

For terrestrial designs, power demand for agitators is determined from a correlation of a dimensionless quantity called power number with the Reynolds Number based on the impeller diameter. Data for this experimentally determined correlation were obtained in terrestrial agitators. The Froude number (the ratio of convectional acceleration to gravitational acceleration) associated with these data was less than 2 in most cases. This correlation cannot be applied without corrections to conditions of microgravity where the Froude numbers are very large. And the corrections must be experimentally determined.

The size of fermenter volume for a given rate of production of cell mass will depend on the mass transfer rate of oxygen through the liquid film which is determined with the help of the quantity, K_1a, which is a product of the mass transfer coefficient and the associated interfacial area. A number of factors determine K_1a which include bubble and cell dimensions, fluid density and rheological properties, agitator and fermenter geometry and power input for agitation. Among these, bubble shape and dimensions, dynamics of bubble movement and hence the gas-liquid interfacial area and the agitator power input are affected by the absence of gravity.

As pointed out by Oldshue[16], $K_{l}a$ does not scale in the same way as reactor size and agitation rate do. The design tools involving correlations of $K_{l}a$ with the other factors must then be recreated for microgravity conditions.

Only two key parameters in the design of fermenters for microgravity application have been discussed above. The purpose was to illustrate a design engineer's concern in having to use terrestrial data and correlations to design space bioreactors. On the other hand, it may turn out that for some of these parameters the corrections for microgravity application are indeed small but it is not possible to know that without conducting experiments specifically for obtaining values of these key parameters in the region of high growth rates of cell mass. It is also necessary to determine experimentally the parametric region where bubble formation, fouling, foaming/entrainment occur under conditions of microgravity.

Once the database for microgravity operation of fermenters is established, a clear and dependable design methodology can be established to design space fermenters in any configuration, size, product. Even if the database is not extensive, it is a great help to the design process to obtain good estimates of the magnitude of corrections to be applied to terrestrial

design data.

TWO CANDIDATE SPACE FERMENTERS

The problem of intimately mixing oxygen with the fermentation broth or separating product gases in the absence of gravity can be overcome by designs that are "gravity independent". Two such design concepts are presented here. In the first, direct gas-liquid contact is eliminated thus obviating the need for dealing with three phase hydrodynamics in microgravity. In the second, the required gas-liquid contact and disengagement are forced in a controllable and predictable manner.

1. The "Gasless" Fermenter

The "gasless" fermenter is a closed sterilizable vessel through which an equally spaced bundle of polydimethyl silicone(PDMS) tubes pass. The PDMS tubes carry the oxygen required for fermentation and the carbon dioxide to be removed from the fermentation broth. Fermentation medium consisting of salts, carbon substrates such as sucrose, and vitamins surround the tubes and fill the vessel. The fermentation fluid can be circulated through an external loop or agitated with an internal marine type propeller to keep uniform con-

centrations inside the vessel.

PDMS is six times as permeable to oxygen as polyfluorosilicone and 25 times as natural rubber and 600 times as high density polyethylene. PDMS preferentially transfers carbon dioxide over oxygen by a factor of 6 to 1. Oxygen diffuses rapidly through PDMS tubes to enter the fermentation broth by dissolution. Silicone hollow fiber tubes are a good choice because they, in addition to permitting high gas transfer rates, exhibit excellent biocompatibility and nonadherence to biological materials as testified by the choice of the same material for human and animal surgical implants. However, due to nonuniformity in tube thickness or tube packing density and in regions of stagnation of the fermentation broth, a small number of oxygen bubbles may form inside the vessel. These bubbles should not be allowed to build up and create problems of direct gas-liquid contact. As a safeguard against this, a small fraction of the fermenter contents will be degassed in a low speed centrifugal separator and the separated liquid returned to the fermenter.

Carbon dioxide passes even more freely than oxygen across the silicone tube walls. However, the removal rate will also be governed by solubility of carbon dioxide in fermentation

broth which again is a function of the pH. Carbon dioxide could be removed from the same tubes containing oxygen, or a portion of the tubes in the bundle may be dedicated for carbon dioxide removal. The use of a carrier fluid such as amines in these dedicated bundles is also a possibility.

Using a 1.5 liter Braun fermenter and a single strand of PDMS tubing, a yeast culture was grown successfully as a preliminary demonstration of this concept at Washington University. Air at 1 atmosphere pressure was supplied to the fermenter through the PDMS tube. The tube outlet was connected to a mass spectrometer gas analysis system which allowed the uptake of oxygen by the fermenter to be measured. The tube occupied 0.08% of the fermenter volume. Transfer of oxygen into water and a fermentation medium as well as sustained yeast growth were demonstrated. Oxygen transfer rate into water at 37C was found to be directly proportional to the oxygen driving force. Transfer of Oxygen into the fermentation was five times slower than into water. An innoculum of yeast (S. cerevisiae) grew to produce a culture with a cell density of about 1g/l in 2 hours. Visual and microscopic examination of the PDMS tubing showed no evidence of fouling after being left in the fermenter for several days.

The "gasless" fermenter was modelled as three CSTR's through which the fermentation broth circulates in cyclic fashion. Through two of these CSTR's PDMS tubes pass carrying oxygen to the broth and carbon dioxide out of it. Monod's model for cell growth was adopted. With this model, a computer program was written at Washington University and the effects of liquid flow rate, gas flow rate, and fraction of total number of tubes dedicated to CO_2 removal. At gas flow rates below 0.5 l/min. dedicating tubes to CO_2 removal actually helped increase the final cell mass. However, above 0.5 l/min. of gas flow all the tubes had to carry oxygen to meet the demand for cell growth. Further, it was found that at flow rates of gas above 1 l/min cell yield does not significantly increase. When five percent of the fermenter volume was dedicated to CO_2 removal tubing a very sizable decrease in dissolved CO_2 concentration was obtained. This result is significant for prevention of bubble formation inside the fermenter.

The absence of direct gas-liquid contact is a unique feature of this design concept eliminating problems of three phase hydrodynamics. This fermenter does not require a gas disengagement volume and since the volume occupied by the silicone tubing is not expected exceed 10%, volume available for cell culture is 90% compared to 60%-70% for conventional fermenters. High oxygen transfer intensities, fewer moving

parts and low shear rates are some of the major advantages of this design. Potential problem areas to be addresses during the design and operation of this fermenter are: Possibility of membrane fouling, regions of stagnation and formation of oxygen bubbles.

2. The Rotating Packed Bed (RPB) Fermenter

The RPB shown in Figure 1 has a cylindrical housing and, proceeding inward, has an annular region for air/oxygen distribution followed by a region of small packings and then a central region for entry of liquid and exit of gases. The whole assembly rotates about the axis of the cylinder. The rotation rate can change the throughput rate or, conversely, for a given throughput rate the mass transfer rate can be changed significantly. The RPB fermenter will not run at high speeds associated with the "Higee" units for fear of disintegration of cell mass. However, the packed bed will provide a more intense renewal of gas-liquid interface that it may be possible to forestall any mass transport limitation on overall cell growth which is an important consideration in the design and operation of fermenters. The radial depth of packing will depend on the space-velocity (or space-time) required to achieve a certain growth rate. Since yeast culture is a low temperature operation, fragile ceramic

packing need not be used. The packing material selected will withstand the high g's developed during STS lift-off.

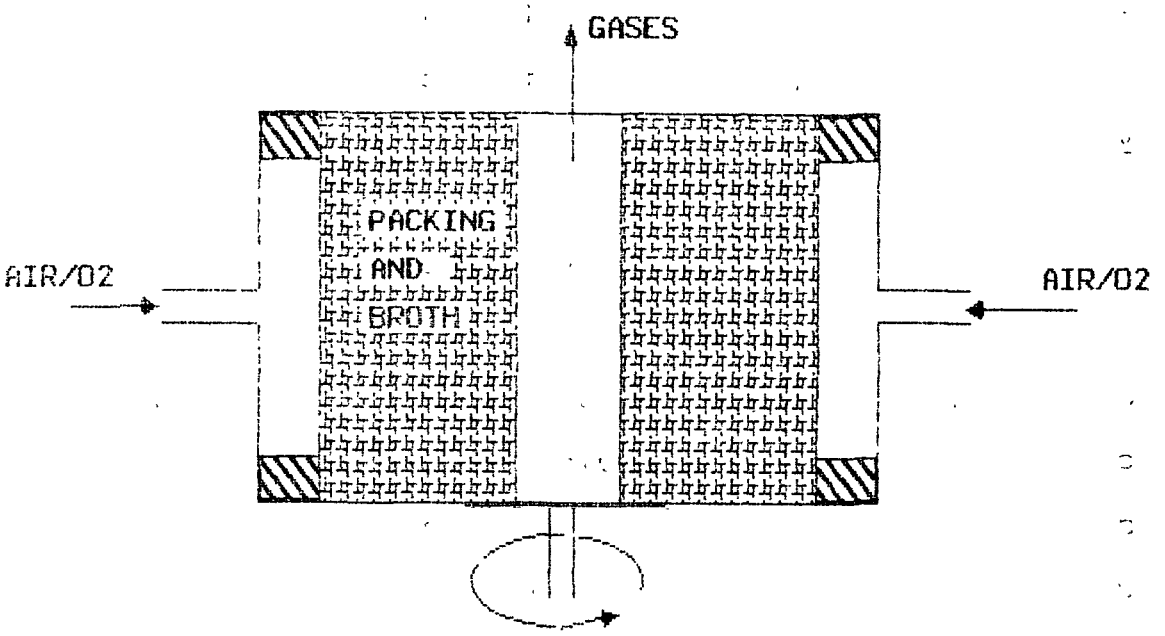

Figure 1. RPB Fermenter

The RPB was adapted from the commercial "Higee" unit of ICI Chemicals. This "Higee" unit was used to intensify chemical separations and its operating principles adapted to micro-gravity use provide a novel fermentation mode that could prove very efficient. Intensification through induced gravity provides a means of dealing with two-phase fermentation. A brief description of the operating principles and how this can be adapted to microgravity fermentation follows.

The Sherwood flooding correlation for packed beds provides a good estimate of the highest gas velocity which can be ob-

tained for a given value of the ratio of liquid to gas flow rates (L/G)[19]. The gas velocity (U) appears in the expression as U^2a/ge^3 where a is the specific area of the packing and e is the packing voidage. The term, g, which normally represents acceleration due to earth's gravity has been recently generalized[18] to mean ambient acceleration to extend the correlation conceptually to other body force fields. When this is done, the correlation opens up new possibilities of packed bed operation through what has come to be called process intensification. For a given value of L/G and the flooding limit provided by the correlation, by decreasing the specific area of the packing, a, or increasing the packing voidage, e, higher gas velocities could be achieved. However, even the latest improvements in packing design could not dramatically increase in gas velocity. Dramatic increases in gas velocity in a packed bed meant correspondingly high shear rates across the gas-liquid interface resulting in a great intensification of interfacial activity. This was indeed absolutely necessary for efficient interphase mass transport. With the announcement of ICI's "Higee" units, the engineering profession became keenly aware of the g-term in the Sherwood correlation which has been taken for granted as a constant to be used to compute the gas velocities correctly. By opening up the possibility that g could be varied by reconfiguring an absorption or distillation unit into a

high speed rotating cylinder packed with much finer packing than could be used before, ICI engineers demonstrated a dramatic reduction in equipment size and weight for a given separation operation. By increasing g in a rotary mode, one had a choice between increasing throughput rates for a given packing size and type or increase the mass transfer rate by enabling the same throughput rate through much finer packing.

The RPB fermenter will not quite operate at the level of intensification achieved by the "Higee" units for two reasons: (1) There is no advantage in supplying dissolved oxygen any faster than the consumption rate of oxygen by the growing cell population, (2) Very high interfacial shear rates obtained in "Higee" units can damage cell walls and terminate fermentation or cell growth process. However, an optimal rotation rate and radial distance will be adopted to take advantage of the intensification effects of centrifugal acceleration while maintaining healthy cell growth. There is another important difference between RPB and Higee units. Higee units are very efficient countercurrent gas-liquid flow generators for rapid separation operations. The RPB fermenter is, on the other hand, a fermentation reactor and not a mere component separator. Therefore, the RPB fermenter is limited in its liquid throughput rate to allow the long space-times required for growth of biomass. In fact, this

fermenter may fall on one extreme end of the Sherwood correlation corresponding to very low liquid throughput rates and very high gas velocities.

The Higee units in operation either in the industry or in the laboratory are exclusively designed and built for separation operations such as distillation and absorption. No Higee unit has yet been built and operated as a chemical reactor or fermenter. A RPB fermenter combines gas-liquid mixing, cell growth and gas liquid separation in one operation with the advantages of lower volume and weight requirements compared to conventional fermenter types. Potential problem areas to be dealt with during design and operation of RPB fermenters are entrainment of liquid and foam by the rapidly counter-current gases.

GROUND AND FLIGHT TESTS

The two fermenter design concepts can be designed and tested in flight and subsequent improvements made following similar flight tests alone and the final design of a particular space fermenter for a definite application determined. In this approach no scale-up factors are obtained and no basic engineering design database is generated. This is a one-shot trial and error approach converging onto an acceptable design.

An alternative is to ground-test and flight-test identical fermenters at two or more sizes with a view to specifically measure mixing effectiveness, bubble sizes, mass transfer coefficients, power required for agitation etc. in addition to growth rate of cell mass. By this approach a sound data base is generated, reliable scale-up factors are derived and procedures for applying corrections to terrestrial designs so as to obtain designs for microgravity conditions.

The design procedure developed will enable the design engineer to calculate, for a given cell mass production rate, optimum gas velocities in a "gasless" fermenter and optimal rotation rates and radial distances and intensification factors in a RPB fermenter. In addition, the design engineer will have the information to guide his choice of fermenter configuration, tubing size and arrangement, degassing requirements, carrier fluids, control of membrane fouling, secondary metabolite production rates and their disposal, extent of foaming, extent of channeling of gases, cell disruption as a function of operating conditions.

CONCLUSIONS AND RECOMMENDATIONS

Microbial food sources are becoming viable and more efficient

alternatives to conventional food sources especially in the context of CELSS in space habitats.

Since bioreactor designs for terrestrial operation will not readily apply to conditions of microgravity, there is an urgent need to learn about the differences. These differences cannot be easily estimated due to the complex nature of the mass transport and mixing mechanisms in fermenters. Therefore, a systematic and expeditious experimental program must be undertaken to obtain the engineering data necessary to lay down the foundations of designing bioreactors for microgravity. This may be the harbinger of a major sub-discipline of variable gravity process engineering.

Two bioreactor design concepts presented here represent two dissimilar approaches to grappling with the absence of gravity in space habitats and deserve to be tested for adoption as important components of the life support function aboard spacecrafts, spacestations and other extra-terrestrial habitats.

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AN ANALYSIS OF THE PRODUCTIVITY OF A CELSS CONTINUOUS ALGAL CULTURE SYSTEM

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One of the most attractive aspects of using algal cultures as plant components for a CELSS is the efficiency with which they can be grown. Although algae are not necessarily intrinsically more efficient than higher plants, the ease with which they can be handled and manipulated (more like chemical reagents than plants), and the culturing techniques available, result in much higher growth rates than are usually attainable with higher plants. Furthermore, preliminary experiments have demonstrated that algal growth and physiology is not detectably altered in a microgravity environment,(1) whereas the response of higher plants to zero gravity is unknown.

In an earlier communication,(2) we described a series of studies on long-term cultures of Scenedesmus obliquus maintained in an annular air-lift column operated as a turbidostat. The primary finding was that productivity vs dry weight rose linearly to a maximum before slowly declining.

In order to rationally design and operate such culture systems, it is necessary to understand how the macroparameters of a culture system, e.g., productivity, are related to the physiological aspects of the algal culture. In this communication we describe a "first principles" analysis of this culture system and derive a mathematical model that describes the relationship of culture productivity to the cell concentration of light-limited cultures. The predicted productivity vs cell concentration curve agrees well with the experimental data obtained to test this model, indicating that this model permits an accurate prediction of culture productivity given the growth parameters of the system.

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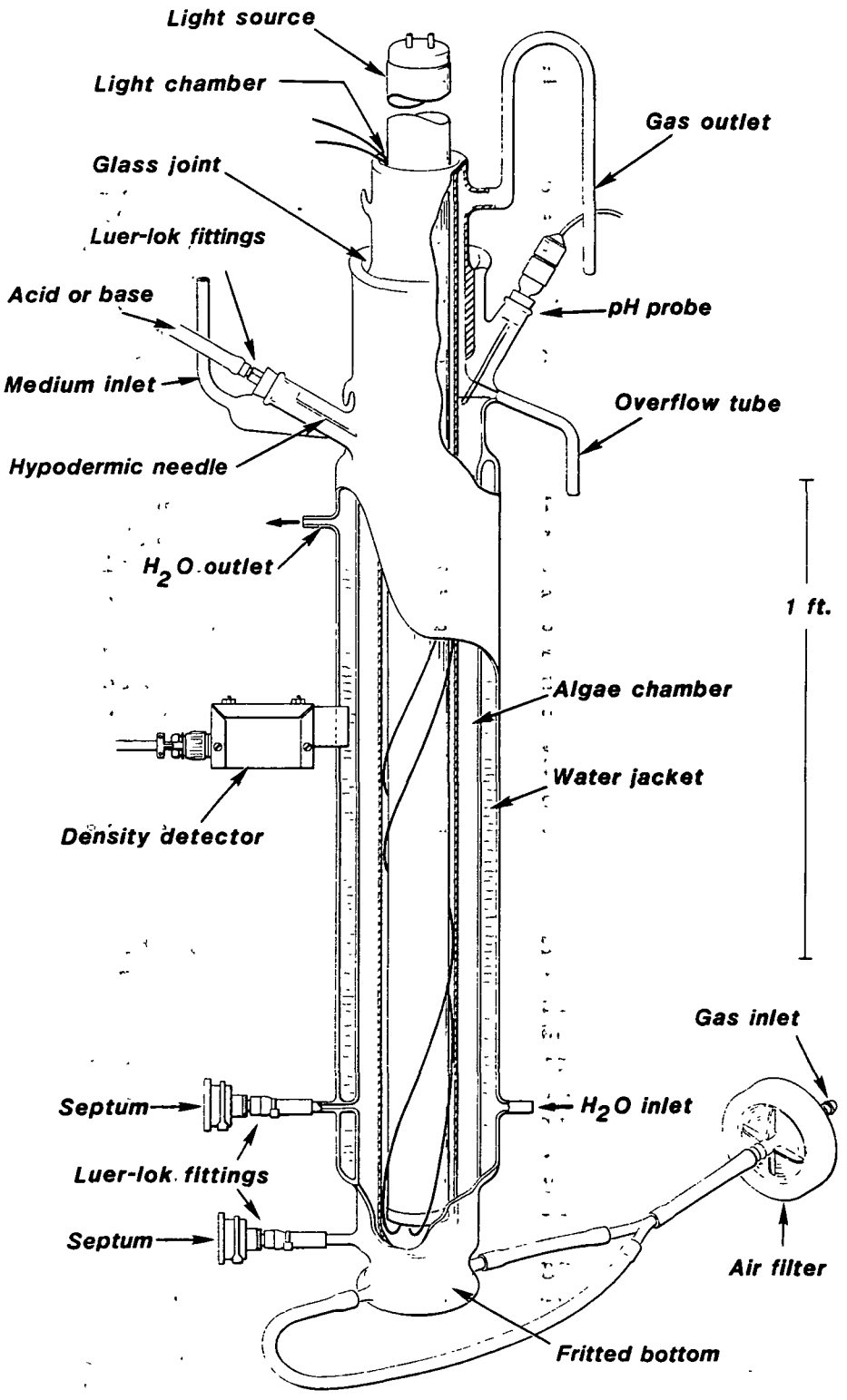

Figure 1 Continuous cell culture apparatus

Materials and Methods

Scenedesmus obliquus (Gaffron strain D_3) was cultured in medium containing 60.0mM KNO_3, 2.0mM $MgSO_4 \cdot 7H_2O$, 1.1mM K_2HPO_4, 0.6mM KH_2PO_4, 68.0µM $CaCl_2 \cdot 2H_2O$, 46.0µM H_3BO_3, 18.0µM $FeSO_4 \cdot 7H_2O$, 12.0µM Na_2EDTA, 9.1µM $MnCl_2 \cdot 4H_2O$, 1.6µM $Na_2MoO_4 \cdot 2H_2O$, 0.8µM $ZnSO_4 \cdot 7H_2O$, 0.3µM $CuSO_4 \cdot 5H_2O$, and 0.2µM $Co(NO_3)_2 \cdot 6H_2O$ and adjusted to pH 6.5. Cultures were bubbled with a gas mixture of 2% CO_2 in air, illuminated with fluorescent light (40W high output, cool white, F24T12), and maintained at 32°C.

The continuous culture apparatus used in this work, shown in Fig. 1, is similar to that described earlier.(2) The apparatus is made of glass and consists of three concentric, cylindrical chambers: the innermost chamber houses the light source, the middle chamber contains the algal culture, and the outer chamber is a temperature-regulated water jacket. Cell density is maintained by monitoring the light transmission through the culture with a photocell. When the output of the photocell exceeds a preset reference value, a microprocessor activates a peristaltic pump, and a defined volume of fresh medium is added to the apparatus. With this system the cell concentration can be continuously maintained within a few percent of a desired value.

Cell density was determined using a Coulter Counter Model TA II with PCA II accessory. Chlorophyll concentration was determined by adding an aliquot of algal culture to a 1:1 mixture of Triton X-100 and 5% KOH in methanol, heating at 63°C for three minutes, and centrifuging. Optical density was determined at 645 nm. Dry weight determinations involved filtering a 10 ml aliquot of algal culture through a glass fiber filter (approximate retention 2.6 µm), rinsing thoroughly with distilled water, drying at 105°C overnight, and cooling to room temperature in a dessicator. [The filter paper was previously dried at 105°C and weighed]. The number of doublings of a culture per 24 hours was determined by measuring the increase in cell density as a function of time. The growth rate of a continuous culture was determined by measuring the volume that overflowed during a defined time period. The productivity ($mg \cdot hr^{-1}$) of a continuous culture is defined as the product of the dry weight ($mg \cdot ml^{-1}$) of the culture and the overflow rate ($ml \cdot h^{-1}$).

Photon flux was measured with a LiCor LI-190SB Quantum Sensor probe; the flux is expressed in units of $\mu E \cdot m^{-2} \cdot s^{-1}$. The extinction coefficient of the algae was calculated by measuring the photon flux in the presence and absence of algae.

Net O_2 evolution was measured polarographically using broad-band saturating, orange-red light (Schott filter OG530 and appropriate heat filters).(3) Oxygen exchange (evolution and uptake) was measured with a quadrupole mass spectrometer, using isotopically labeled O_2 (98 atom% [$^{18}O_2$], Cambridge Isotope Laboratories). The instrumentation and methods for data analysis and calibration were as described previously (4), except that the data were stored on magnetic tape and processed by a Sym-1 microcomputer. Illumination for these measurements was supplied by fluorescent bulbs having a photon flux comparable to that used to grow the culture. All experiments were performed at 32°C.

Derivation of Model Equation

The productivity Y of a photoautotrophic algal culture will be equal to its rate of photosynthesis (P) minus its rate of respiration (R), i.e., $Y = P - R$. Ideally, for a fixed incident light intensity the total photosynthetic yield of the culture would increase asymtotically with increasing cell concentration. However, this increase in photosynthesis with increasing cell concentration would be counterbalanced by an increase in the total respiration of the culture. Therefore, one would predict that a plot of the overall productivity of the system as a function of cell concentration will go through a maximum.

Under conditions in which the photosynthetic apparatus is not light saturated, the maximum rate of photosynthesis of a culture, i.e., when every photon of light is absorbed, is given by the expression $E_m I_o AK$, where E_m is the maximum theoretical conversion of the energy of the absorbed light into chemical energy by a photosynthetic system, I_o is the light intensity available to the culture, A is the illuminated area of the culture, and K is a conversion factor for the energy equivalent of the algae. In cases in which not all of the available light is absorbed, the actual rate of photosynthesis will be the maximum rate minus a term that describes the amount of light

transmitted (i.e., not absorbed) by the culture. The expression describing the amount of light transmitted is a form of Beer's Law,(5) in which the transmitted light intensity I, is equal to $I_o e^{-\varepsilon cl}$, where ε is the extinction coefficient, c the cell concentration, and l the path length. Therefore, the rate of photosynthesis of the culture is given by:

$$P = E_m I_o AK - E_m I_o AKe^{-\varepsilon cl} \qquad (1)$$

or

$$P = E_m I_o AK\,(1-e^{-\varepsilon cl}) \quad . \qquad (2)$$

The respiration term R' is the product of the cellular respiration rate, R, the cell concentration, c, the total culture volume, V, and G, a term related to the amount of carbon per cell. Therefore, the productivity of the algal culture can be defined by:

$$Y = E_m I_o AK\,(1-e^{-\varepsilon cl}) - GRcV \qquad (3)$$

where

Y	=	Yield $[(g\ cells)\cdot hr^{-1}]$
E_m	=	0.2, the maximum attainable photosynthetic conversion on an energy basis
A	=	Illuminated area (m^2)
K	=	0.156 $[(g\ cells)\cdot hr^{-1}\cdot watt^{-1})]$, the energy equivalent of the algae
I_o	=	Light intensity $(watts\cdot m^{-2})$
ε	=	Extinction coefficient $(l\cdot cm^{-1}\cdot g^{-1})$
c	=	Cell concentration $(g\cdot l^{-1})$
l	=	Light path (cm)
R	=	Respiration rate $[g\ carbon\cdot(g\ cells)^{-1}\cdot hr^{-1}]$
V	=	Culture volume (l)
G	=	Ratio of g cells to g carbon (2.04).

For the culture apparatus described in Fig. 1, the illuminated area is 0.1471 m^2, the culture volume is 1.2 l, the light intensity is 12.9 $W\cdot m^{-2}$ (59.6 $\mu E\cdot m^{-2}\cdot s^{-1}$), and the light path is 1.25 cm. The cells were 49% carbon, so that G = 2.04. The value for K (0.156 g $cells\cdot hr^{-1}\cdot W^{-1}$) was determined calorimetrically,

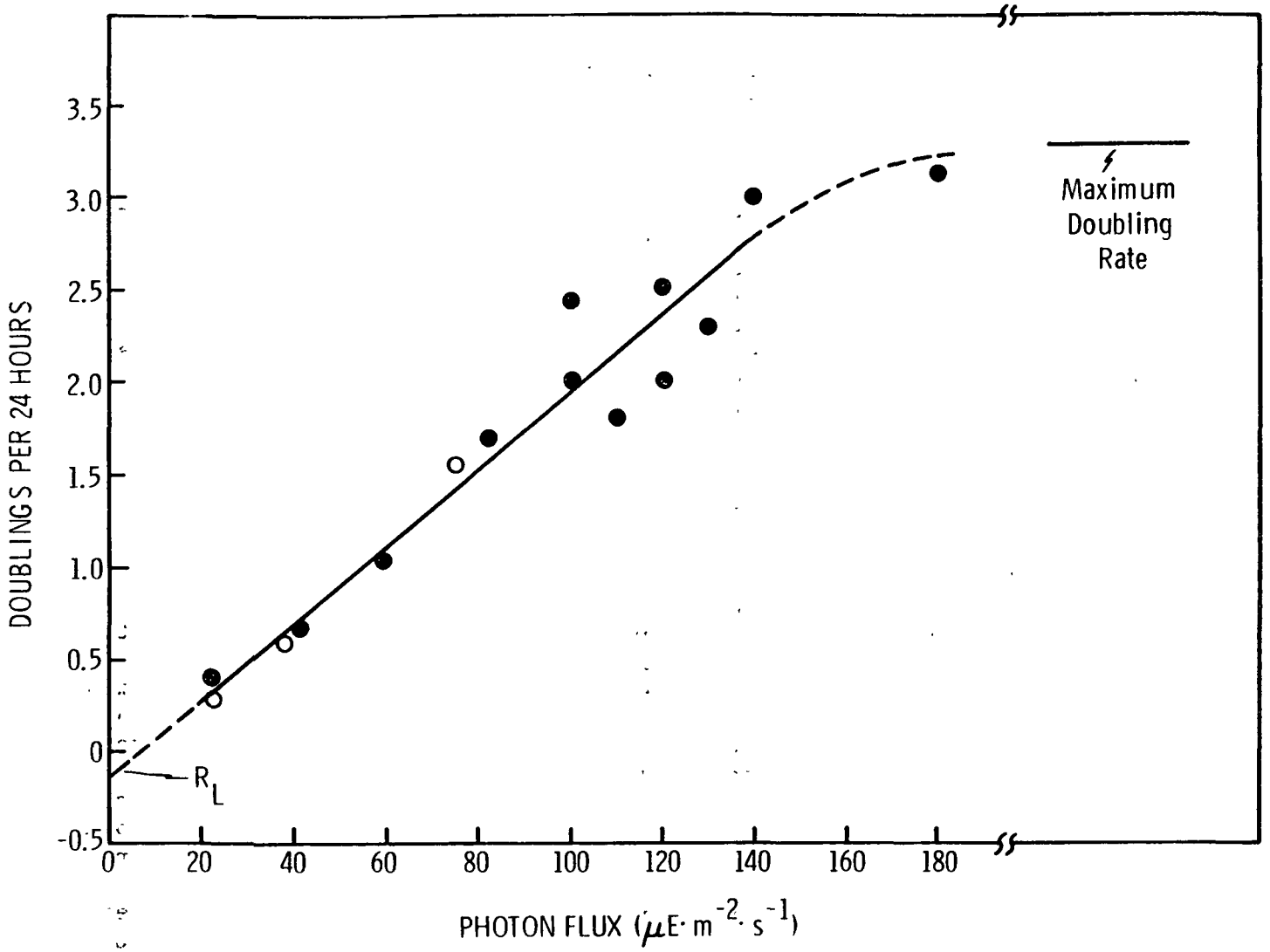

Figure 2 Growth rate (expressed as doublings·day^{-1}) of Scenedesmus as a function of photon flux in Roux culture bottles (closed circles) and continuous culture (open circles). R_L equals respiration rate extrapolated from the light curve.

and agrees with published values.(6) The maximum attainable photosynthetic conversion E_m was taken to be 20%.(7)

The above analysis assumes that 1) photosynthesis is proportional to the amount of absorbed light, i.e., light is not saturating, a necessary condition for efficient light utilization, and 2) the rate of respiration R is a constant or known function of I and c. In addition, the absorption coefficient ϵ is not a constant with respect to cell concentration, and thus the relationship between ϵ and c must be ascertained. These items are considered further in the following sections.

Results and Discussion

Light Limitation

Figure 2 shows the relationship between photosynthetic growth of Scenedesmus and photon flux using culture (Roux) bottles (closed circles) and our continuous culture system (open circles). The doublings per 24 hours increase monotonically with photon flux up to approximately 140 $\mu E \cdot m^{-2} \cdot sec^{-1}$ at which point the growth rate approaches the maximum. Note that there is very good agreement between the growth rate obtained in the continuous culture and Roux bottle growth systems. At the highest photon flux of the continuous culture system (75 $\mu E \cdot m^{-2} \cdot s^{-1}$), the growth rate is still on the linear portion of the curve. This finding indicates that the algae in the continuous culture system are not light saturated and that photosynthesis is proportional to the amount of absorbed quanta. Therefore, a necessary condition of the derived model is satisfied.

Extinction Coefficient

Figure 3 shows the relationship between the integrated (400-700 nm) average absorption coefficient (ϵ) and cell concentration in the culture system. Note that this relationship is neither constant nor linear, reflecting the fact that at high cell densities the predominant green light is only poorly absorbed. This phenomenon has been quantitatively rationalized in terms of Beer's Law, which is strictly valid only for monochromatic light.(5)

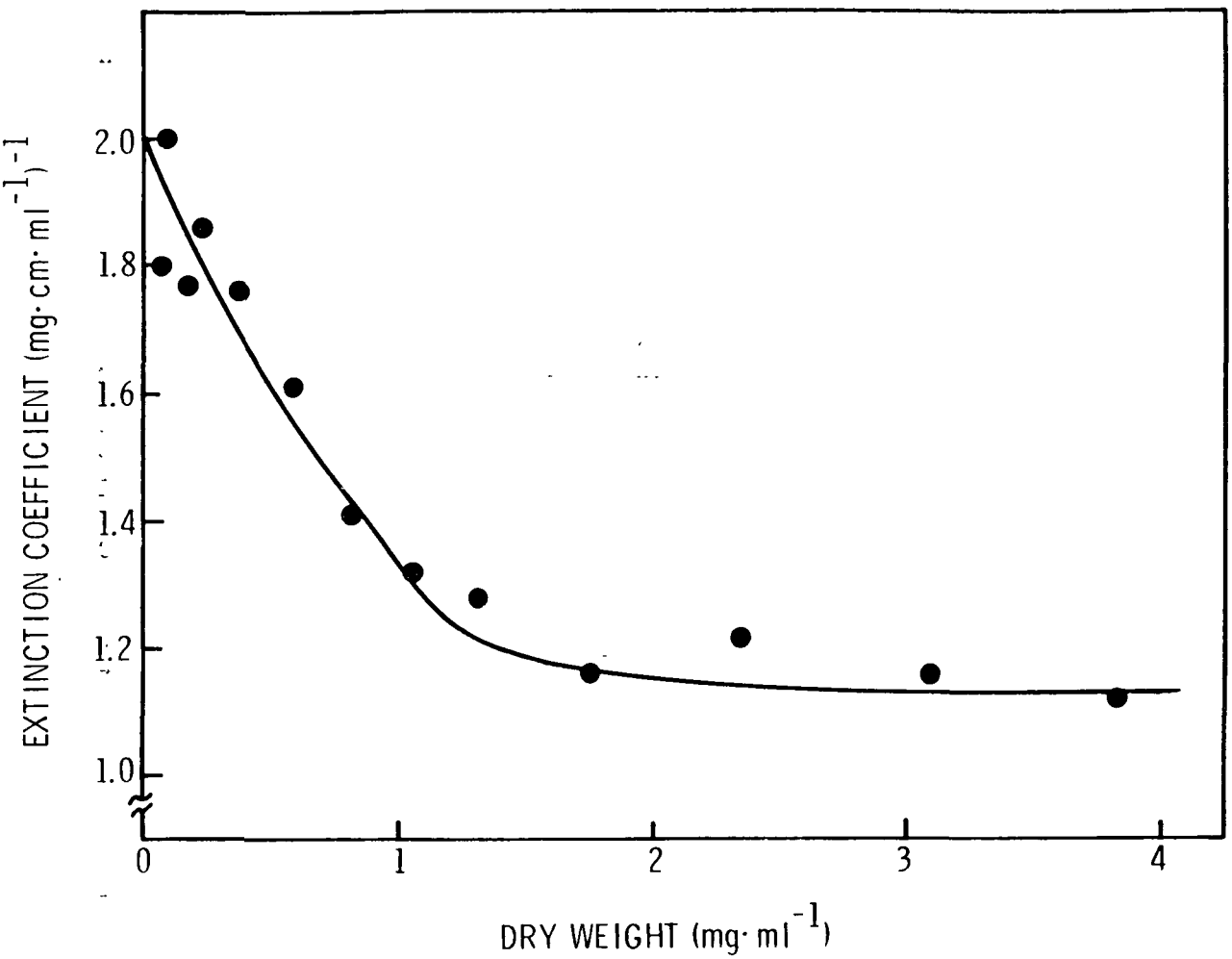

Figure 3 Integrated (400-700 nm) absorption coefficient of Scenedesmus as a function of cell density (expressed as g dry wt·ml^{-1}).

Cellular Respiration

Because it is difficult to accurately measure respiration in a photosynthesizing system, we used three different approaches. In the first, we extrapolated the data from Figure 2 to zero light flux to measure the respiration rate, R. In this case, the growth rate at zero light is -0.12 doubling per 24 hours, which corresponds to a respiration rate of 4.1 μmoles CO_2 evolved·mg $chl^{-1}\cdot hr^{-1}$ or 0.0024 g carbon·g $cells^{-1}\cdot hr^{-1}$.

The second approach involves measuring oxygen consumption of a darkened culture using an oxygen polarograph. As shown in Fig. 4, thoroughly dark-adapted cells had a low respiration rate, ca. 2.8 μmoles O_2 consumed·mg $chl^{-1}\cdot hr^{-1}$ (0.0016 g carbon evolved·g $cells^{-1}\cdot hr^{-1}$). When illuminated, the respiration rate was masked by a high rate of photosynthetic oxygen evolution. Immediately after the cessation of illumination, there was a high rate of respiration (13.9 μmoles O_2 consumed·mg $chl^{-1}\cdot hr^{-1}$ or 0.0081 g carbon evolved·g $cells^{-1}\cdot hr^{-1}$) which, after ten minutes in the dark, declined to about the same rate as before illumination. High respiration rates immediately after illumination have been previously observed.(8) Although these observations are not new, these values provide limits for the respiration rate needed for the mathematical model.

Figure 5 shows the results of an experiment in which a specially constructed mass spectrometer inlet system was used to measure the cellular respiration rate <u>during</u> illumination. The mass spectrometer can measure the concentration of two different oxygen isotopes ($^{16}O_2$ and $^{18}O_2$) simultaneously in solution. Therefore, this third approach provides a means to distinguish between respiratory oxygen consumption and photosynthetic oxygen evolution. The experiment in Fig. 5 was performed under the same conditions used for continuous culture apparatus; thus, the oxygen exchange (consumption and evolution) should closely mimic that occurring in the culture system. Upon illumination, there was an initial burst of oxygen uptake that subsequently leveled off and reached a steady state that corresponds to 8.5 μmoles O_2 consumed·mg $chl^{-1}\cdot hr^{-1}$ (0.0049 carbon evolved·g $cells^{-1}\cdot hr^{-1}$). Concurrently, there was a sustained, light-dependent rate of oxygen evolution (60 μmoles O_2 evolved·mg $chl^{-1}\cdot hr^{-1}$), a value consistent with the calculated growth rate based on the productivity of the culture.

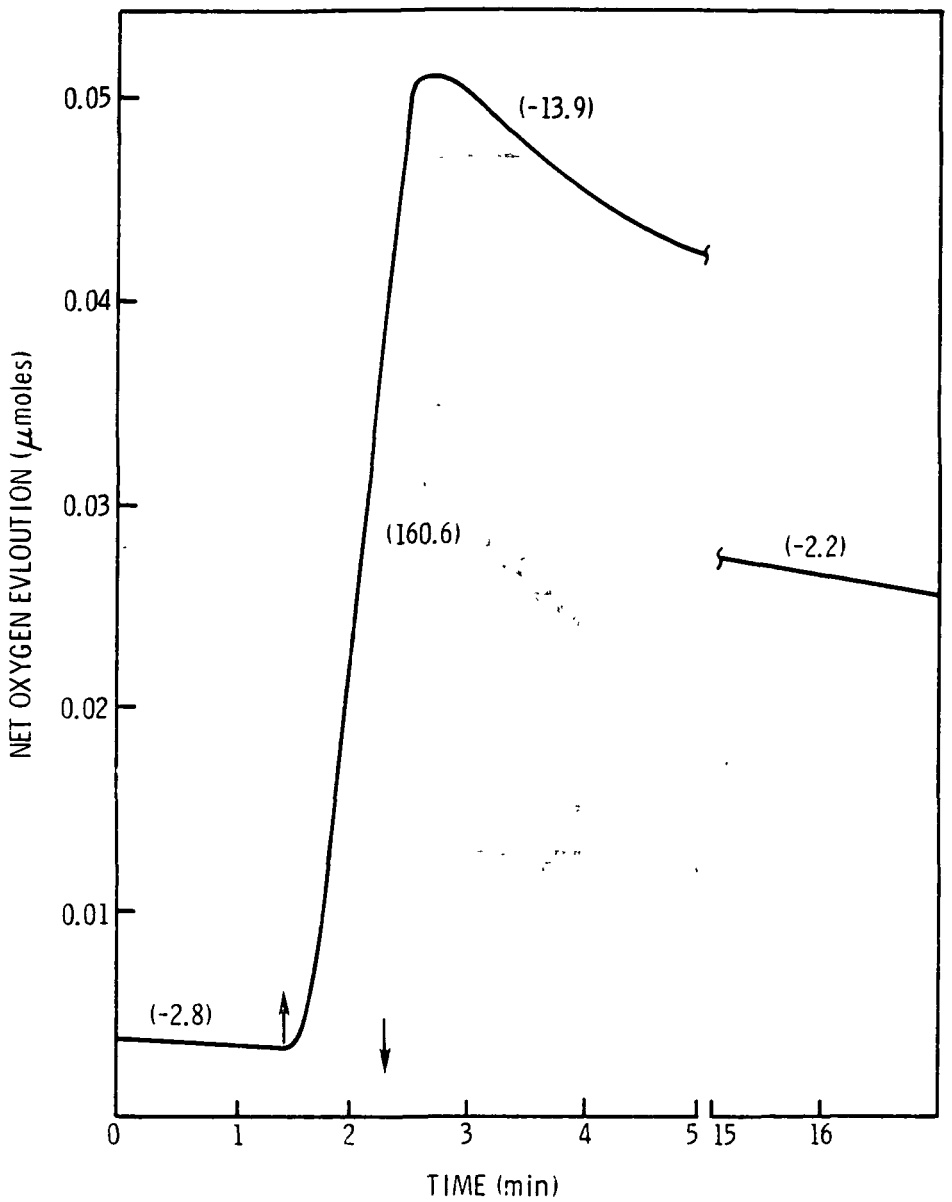

Figure 4 O_2 trace obtained when dark-adapted (>10 min) Scenedesmus was illuminated (↑) and subsequently darkened (↓). Values in parentheses are computed O_2 exchange rates (in units of μmoles O_2 • mg $chl^{-1} \cdot hr^{-1}$).

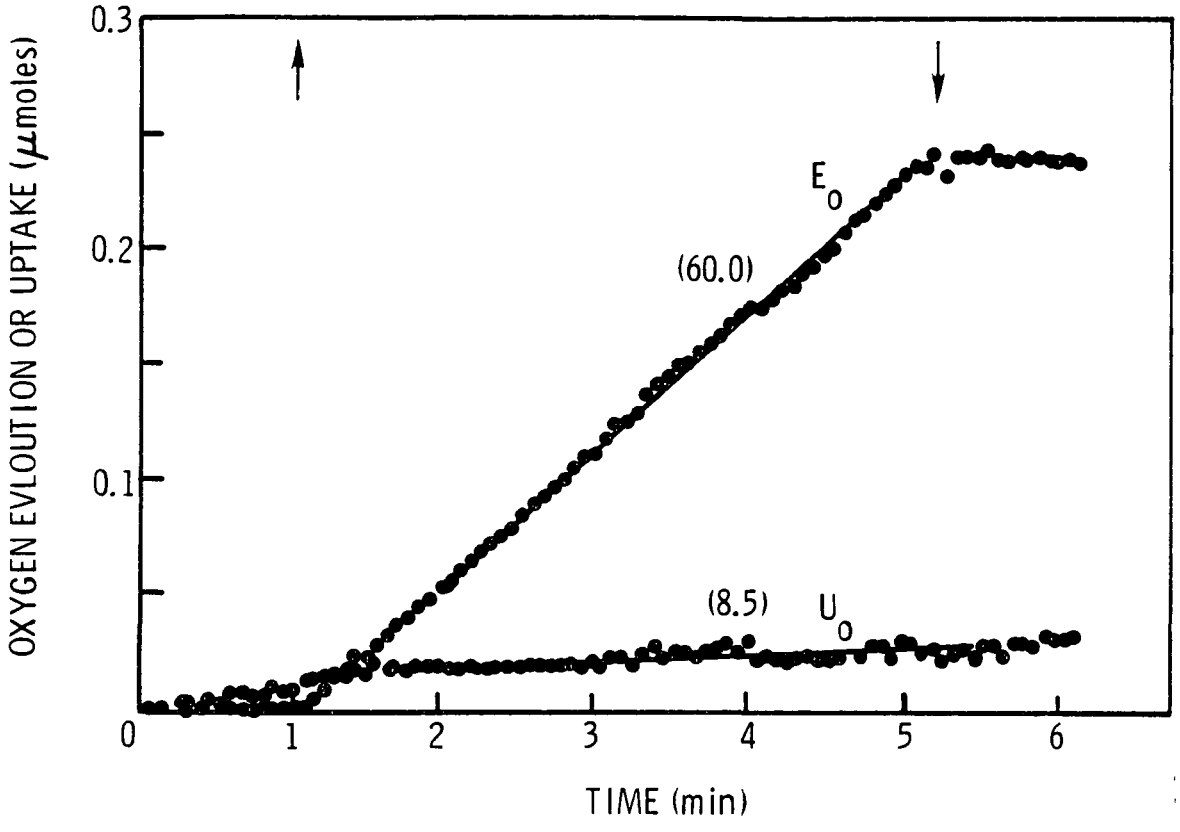

Figure 5 Computed time courses of O_2 evolution (E_o) and O_2 uptake (U_o) obtained using a mass spectrometer system to monitor the gas exchange of Scenedesmus. ↑ and ↓ indicate light-on and light-off, respectively.

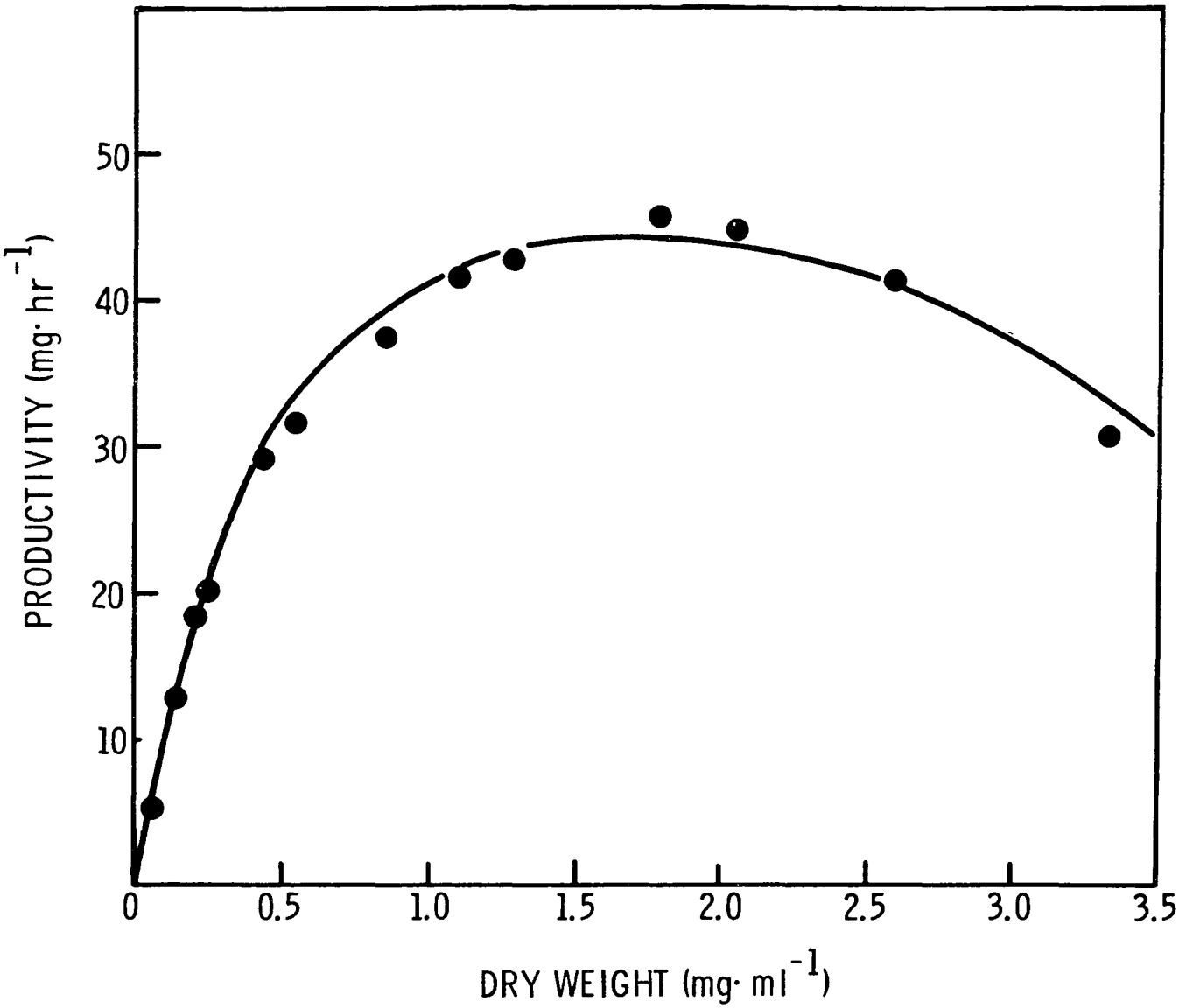

Figure 6 Relationship between culture productivity and cell density (measured as dry weight) in the continuous culture apparatus shown in Fig. 1. The closed circles show the experimental data obtained. The solid line shows the relationship predicted using the model described in the text.

Each of the three independent analytical methods used to estimate the cellular respiration rate has particular aspects that limit its accuracy for determining the respiration rate during illumination. However, together they can provide an approximation, which, in this case, is 5.0 μmoles $O_2 \cdot mg\ chl^{-1} \cdot hr^{-1}$ (0.0029 g carbon evolved$\cdot$g cells$^{-1}\cdot$hr^{-1}). This value will be used in the model equation.

Comparison of Observed Productivity to Theoretical Predictions

Figure 6 illustrates the observed and predicted relationships between culture productivity and dry weight. The data points were obtained in a series of experiments in which Scenedesmus was maintained in the continuous culture system. The solid line is the relationship predicted using the values assigned and derived in the previous sections. The excellent agreement between the observed and predicted values suggests that the model accurately describes the culture system in terms of physiological parameters.

At cell concentrations greater than about 1.5 mg$\cdot$ml^{-1}, the relationship between extinction coefficient and cell concentration is approximately constant (Fig. 3). Thus, between these limits we can differentiate Eq. 3 with respect to c. Under these conditions, maximum productivity will occur at a cell concentration of:

$$c_{mp} = \frac{1}{\varepsilon 1} \ln \frac{GRV}{\varepsilon 1 E_m I_o AK}$$

where c_{mp} is the cell concentration at maximum productivity. If we use the parameter values given above, we compute that c_{mp} = 1.73 mg$\cdot$ml^{-1}, which agrees well with the data of Fig. 6.

We should emphasize that this model is completely determined and has no free adjustable parameters. Its main limitation appears to lie in the experimental determination of the respiration rate, R. Although the model accurately describes the growth of photoautotrophic algae in this system, it is limited to cases in which all growth factors except light are present in excess.

We are presently working to modify this model to account for conditions where CO_2 concentration is limiting for cell growth.

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THE DEVELOPMENT OF AN UNCONVENTIONAL FOOD REGENERATION PROCESS: QUANTIFYING THE NUTRITIONAL COMPONENTS OF A MODEL METHYLOTROPHIC YEAST

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INTRODUCTION

Closing the food loop for extended space missions is a function of the economics of transportation versus regeneration. The determinants of that function are 1) the weight/volume requirements and 2) the suitability or palatability of the food produced. The latter determinant is so compelling as to limit the number of choices for food regeneration to largely conventional plant agronomy. However, the need for system redundancy, protein supplements and production efficiency argues for the inclusion of unconventional food regeneration schemes. (1,2) This use of unconventional food production becomes especially tenable given the highly developed state of food processing which can render almost any basic food commodity palatable. In addition, if the weight/volume factors can be shown to be advantageous for unconventional food regeneration, then these routes will be useful from the economic standpoint.

Work in our laboratory at JPL has focused on a hybrid

chemical/biological approach to unconventional food regeneration. Carbon dioxide and water, the major wastes of human metabolism would be converted to methanol by one of several physico-chemical processes available (thermal, photocatalytic, etc.). Methanol is then used to supply carbon and energy for the culture of microorganisms which in turn produce biologically useful basic food stuffs for human nutrition. Our work has focused on increasing the carbohydrate levels of a candidate methylotrophic yeast to more nearly coincide with human nutritional requirements. Yeasts were chosen due to their high carbohydrate levels compared to bacteria and their present familiarity in the human diet. The initial candidate yeast studied was a thermotolerant strain of Hansenula polymorpha, DL-1. This paper describes the quantitative results that permit an evaluation of the overall efficiency in hybrid chemical/biological food production schemes. A preliminary evaluation of the overall efficiency of such schemes is also discussed.

DETERMINING THE NUTRITIONAL PROFILE OF H. POLYMORPHA

Table I gives a summation of data from an earlier report and shows the carbohydrate profile of the methylotroph and the common yeast, Saccharomyces. (3). This data also provides the necessary information required to evaluate the conversion efficiencies of the proposed approaches. Obtaining the analytical data required the adaptation of available procedures and the development of new approaches. The methods involved

the use of oxido-reduction analyses, enzymatic analyses, and difference calculations. The total CH_2O, protein, and glycogen analyses were obtained by direct analysis of whole cell preparations. The glycogen assays were most difficult to obtain and required significant efforts to correlate qualitative and quantitative data. (3-5) The table shows mass balance calculations. These were made to confirm the validity of the results. Some cellular components needed to be estimated such as nucleic acid, unhydrolyzed polysaccharides, lipids and ash. Reasonable estimates of these fractions were made using published data and other experimental data. Strains of Saccharomyces were also analyzed to provide comparative data. An examination of the data in Table I reveals that the data are accurate based on mass balance calculations. An additional confirmation of this data comes from earlier qualitative data which correlates closely with the data in Table I and the independent analysis of protein and carbohydrate values given by Johnson Space Center's Biomedical Laboratories (references 4, 5 and Table I).

An initial premise was made to treat this microbiological food regeneration scheme as a primary food source. Since the edible carbohydrate level was naturally low in aerobically grown yeasts (see Table I wild type H. polymorpha and Saccharomyces), strains of H. polymorpha were selected which had enhanced levels of the intracellular storage carbohydrate,

TABLE I

Composition Profile of H. polymorpha and Saccharomyces

	FRACTION OF DRY WEIGHT (%)							
	H. polymorpha					Saccharomyces		
ANALYSIS FOR	DL1	#13	#122	#84	#84[a]	S. cerevisiae	S. uvarum	S. diastaticus
TOTAL CH_2O	69.8*	69.6	65.5	70.8	75.2	42.2	27.5	33.2
Glycogen	6.5	14.0	0.0	20.0	-	2.9	-	-
Mannan	39.9	32.9	42.5	26.0	-	26.8	-	-
Trehalose & Acid Soluble ω-Glucan	23.4	22.7	23.0	24.8	-	12.5	-	-
PROTEIN	17.0*	24.4	20.9	12.5	13.7	48	58	47
CH_2O + Protein	86.8	94.0	86.4	83.3	88.9	90.2	85.5	80.2
Other Cellular Components (Estimated)	10	10	10	10	10	8	8	8
MASS BALANCE TOTAL	96.8	104	96.4	93.3	98.9	98.2	93.5	88.2

[a] Data from Johnson Space Center Biomedical Laboratory. This analysis from JSC Biomedical Laboratory also provided an actual value for fat and fiber of 1.2% and 0.8%, respectively.

glycogen. Table I shows that strains were selected that had two to three times as much glycogen as the parent strain (#13 and #84).

EVALUATING CONVERSION EFFICIENCIES

The conversion efficiency of methanol to edibles can be determined directly from the data obtained in Table I. Table II shows the data from Table I redisplayed by substate source into the edible fractions, protein and carbohydrate. The carbohydrate fraction is the sum of glycogen and trehalose fractions (trehalose is estimated by a difference procedure described in reference 3). The data is shown for H. polymorpha and three of its mutant strains (one strain was a glycogen deficient strain used as in internal control, #122) and three strains of the common yeast Saccharomyces. The Saccharomyces strains were not grown on limited nitrogen as were the methylotrophs and the lower carbohydrate levels are indicative of this nutritional difference. Total edibles for the methylotrophic strains approaches the glucose grown strains only in the two high glycogen mutants. The ratio of protein to carbohydrate is roughly reversed for the Saccharomyces strains.

These data can be converted into a carbon efficiency if the levels of methanol consumed can be correlated with total

TABLE II

Edible Fractions of Hansenula Polymorpha

and Saccharomyces Cerevisiae

		Fraction of dry weight (as %)		
Substs	YEAST SPECIES	EDIBLES		TOTAL EDIBLES
		PROTEIN	CARBOHYDRATE	
	H. polymorpha			
	Wild type	17.0	27.9	44.9
Me	#13 (High glg)	24.4	34.7	59.1
	#84 (High glg)	12.5	44.8	57.3
	#122 (Low glg)	20.9	21.0	41.9
	S. cerevisiae*	48.0	13.4	61.4
	S. uvarum	55.0	20.4	75.4
Glu	S. diastaticus	47.0	21.2	68.2

* Approved by FDA for human consumption

cell mass produced. This is easily done using gas chromatographic analysis of growth media for methanol and using gravimetric determinations of the cell mass as a function of time. Typical conversion to cell mass has been reported at 35% for autotrophs (6). In other experiments in our laboratories with the methanol grown strain, Hansenula capsulata, conversion of methanol to cell mass was measured at 30-35% in early stationary phase (data not shown). Thus a 30-35% conversion of methanol to cell mass was considered reasonable for H. polymorpha. Using the data from Table II and this estimated conversion efficiency, the overall yield for edible food regeneration in this methanol grown system can be calculated to be 16-21%. For the glucose grown Saccharomyces strains, the values are about 5% higher at 21-26%.

Based on this data it is possible to estimate a total overall conversion efficiency of a hybrid chemical/biological regeneration process if the conversion of CO_2 to methanol can be estimated. A reasonable estimate can be made by considering the individual known and estimated conversion efficiencies involved in the splitting of water to H_2 and O_2, the reduction of CO_2 to CO, and the reduction of CO to methanol by H_2. Using photocatalytic and photoelectrocatalytic processes, an estimate of 32% conversion from solar energy to methanol can be made with reasonable confidence (V. Miskowski and S. DiStefano, personal communication). Combining this with the biological

efficiencies estimated above, an overall conversion efficiency for this candidate hybrid chemical/biological system was calculated to be 5.1-6.7% (using 35% as the biological value).

SIGNIFICANCE

A preliminary evaluation for a candidate foor regeneration system has been accomplished. The system involving CO_2 reduction to methanol, followed by yeast growth on the methanol to produce protein, edible carbohydrate and lipids appears to be a worthy candidate for further development. We have been able through genetic modifications and specific cultural conditions to increase the content of digestible carbohydrates in a methylotrophic yeast to better meet human dietary requirements.

The energy efficiency calculations also suggest that the system is at least on a par with conventional agriculture and may have some significant advantages over such food regeneration. While many factors must be considered in final selection of food regenerating systems, the hybrid system described herein merits consideration for further development. If not this specific system, then at least the "class" of systems employing chemical reduction of CO_2 and water to simple organics, and the utilization of microorganisms growing on these reduction products to produce human food nutrients, should be considered in any overall food regeneration system in CELSS.

FUTURE EFFORTS

Efforts are underway to subject another methylotroph and an FDA-GRAS approved yeast strain (Candida boidinii and Candida utilis, respectively) to the same types of analyses to further validate the approach and to provide a larger body of data on possible candidates for unconventional food regeneration schemes.

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Application of Photosynthetic N_2-fixing Cyanobacteria to the CELSS Program

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Abstract

Certain cyanobacterial species have the unique ability to reduce atmospheric N_2 to organic nitrogen. These organisms combine the ease of cultivation characteristic of prokaryotes with the fully developed photosynthetic apparatus of higher plants. This, along with their ability to adapt to changes in their environment by modulation of certain biochemical pathways, make them attractive candidates for incorporation into the CELSS program.

A high percentage of the cyanobacterial biomass is in the form of protein, much of which exists in discrete high molecular weight granules (phycobilisomes and carboxysomes). We have shown that with proper manipulation of the osmotic environment, high amounts of granular glycogen can also be produced. These particular fractions, along with the ability of certain species to aggregate, may negate future need for conventional harvesting and fractionation techniques, such as high speed centrifugation.

We are undertaking a study, using commercially

available air lift fermentors, to simultaneously monitor biomass production, N_2-fixation, photosynthesis, respiration, and sensitivity to oxidative damage during growth under various nutritional and light regimes, to establish a data base for the integration of these organisms into a CELSS program.

Introduction

The use of biological components in the CELSS program as subsystems for the revitalizaton of air, waste processing, and for the production of food has been proposed for long-term space flight[1]. To this end, many systems of biomass (protein, carbohydrate, lipid) production are under consideration by NASA. Employment of photosynthetic systems (higher plants, green algae and cyanobacteria) allow biomass production from relatively simple components which are readily recycled in a CELSS system, namely CO_2, minerals (NO_3^-, PO_4^-, K^+, Na^+, etc.) and micronutrients.

The production of plant material in a closed system presents several problems, two of which we shall address here. A primary consideration is that of the energy requirement for continuous operation, including illumination, temperature control and various maintenance mechanisms. One way in which the energy demands can be lessened is by modifying the quality of the light used. Illumination of photosynthetic systems with white light

(300-700nm range) is wasteful, since photosynthetic organisms utilize only selected regions of the visible spectrum. Tailoring the emission profile of the light source to match that of the action spectrum of photosynthesis, or to select an "emission band" in a region of maximal absorption by some of the photosynthetic pigments would present an energy saving, especially if the selected "emission band" was in the blue region, when using fluorescent light sources.

A second concern is the probability of nitrogen loss from the closed system by the action of contaminating denitrifying bacteria, which degrade NO_3^- to N_2 gas, which is then lost from the biological system[2]. Denitrification is most likely to occur irrespective of the method of waste processing due to the storage of human waste and non-food biomass (cellulose), which would result in an irreversible loss of biologically essential nitrogen.

To address the above problems, we have proposed the use of cyanobacteria (blue-green algae) as the photosynthetic organism of choice.

Cyanobacteria occupy a unique position in the hierarchy of plant form and structure. Like higher plants, they carry out the O_2 evolving photolytic cleavage of water and the subsequent fixation of CO_2 into carbohydrate. Unlike higher plants, however, during periods of

nitrogen starvation (when NO_3^- levels in the medium are depleted) certain species differentiate specialized cell types (heterocysts) which maintain low oxygen tension, a prerequisite for their major function: the reduction of atmospheric N_2 to NH_3, prior to its incorporation into amino acids and proteins. In addition, cyanobacteria possess prokaryotic genetics, which allow the future possibility of genetic manipulation. Also, environmental factors have been shown in certain strains to modify the partitioning of photosynthetic reductant to certain macromolecules[3,4] and storage carbohydrate[5]. These factors make the cyanobacteria prime candidates for consideration in the CELSS program, as either a primary source of biomass or as a supplement to other algal/higher plant subsystems, to reverse the effect of denitrifying bacteria, and recycle atmospheric N_2 back into the biological systems.

Research Objectives

Due to the suitability of cyanobacteria for integration as a CELSS subsystem, we propose to investigate biomass production in the form of dietary compatible macromolecules (protein, lipid, carbohydrate) of nitrogen fixing heterocystous cyanobacteria in (commercially available) air-lift type fermentors. The research plan is to investigate environmental effects of nutrient supply, particularly CO_2 (the major limiting factor for plant

growth) and the effects of various light regimes on productivity, in particular where it affects N_2 fixation. Due to the more efficient conversion of UV photons to photons in the blue region rather than to the full visible range by fluorescent discharge tubes, we shall investigate growth characteristics, biomass production, and certain relevant biochemical functions (photosynthetic O_2 evolution, respiration, and nitrogen fixation) on cells grown under blue light, compared to an equal concentration of white light photons. The blue "emission band" overlaps absorption bands of the light harvesting antennae pigments chlorophyll and phycobiliproteins.

One of the major factors affecting nitrogenase activity is the ambiant oxygen level. Although the heterocysts possess a thick cell wall which slows O_2 diffusion to rates that are compatible with the rates of O_2 removal by the respiratory system, it is quite likely that intracellular levels of O_2 (particularly in the cells directly adjoining the heterocysts) may exceed usual environmental levels. To determine the role of O_2 in such a system, we have been developing an electron spin resonance (ESR) method which monitors the broadening of a paramagnetic spin probe molecule by interaction with O_2, which is itself a paramagnet in the ground state. Interaction of the spin probe with other paramagnetic species causes a measurable broadening of the probe's ESR signal line width. Since the probe can be completely broadened

by adding a cell membrane impermeable paramagntic reagent, the broadening due to O_2 inside of the cell can be monitored. Using this technique, we plan to investigate the effect of cellular O_2 levels, where this may influence the rates of nitrogen fixation.

Materials and Methods

Nostoc muscorum (Anabaena #7119) was grown in BG11 medium minus nitrate in a 2 litre Bethesda Research Laboratories Airlift Fermentor at 28°C, 150 u $Es^{-1}m^{-2}$ light (using Bethesda Research Laboratories 2201 LB day light white 300-700 nm, or 2201 LF blue light 400-500 nm) with an air flow rate of 2 litres/min, supplemented with 0.5% CO_2 . 200 ml aliquots were withdrawn daily, the fermentor volume made up by addition of 200 ml of sterile medium. Cells were centrifuged at 10,000xg/10 min and resuspended to 10 ml in BG11 medium suplemented with 10 mM Tes buffer pH 7.0.

O_2 evolution was monitored polarographically in BG11 plus 10mM Tes pH7.0 with a cell density equivalent to 1-2 ug chlorophyll/ml. Nitrogenase activity was determined in whole filaments by monitoring acetylene reduction using a Varian Model 3700 gas chromatograph fitted with a Poropak T column. Cells (3 ug chlorophyll in 3 ml) were assayed in a 5 ml vial under air plus 10% (v/v) C_2H_2 in a shaking

Table I

CYANOBACTERIA -- (BLUE-GREEN ALGAE)

Organism	Comments	Rate of N_2 Reduction umoles.mg chlorophyll.hr	Reference
Anabaena sp. ATCC 29151 PCC 7119 (Nostoc muscorum)	filamentous heterocystous freshwater 26°C, 2000-3000 lux	5-10	Stewart, W.D.P., 1977; Blumwald, E., and Tel-Or, E., 1982
Anabaena sp. ATCC 33047 (Sea anabaena)	filamentous heterocystous marine 37°C, 1600 lux	30-60	Stacey, G. et al., 1977
Gloeothece sp. ATCC 27152 PCC 6909 (Gloeocapsa sp.)	unicellular non-heterocystous freshwater 26°C, 2000-3000 lux	2-4	Kallas, T. et al., 1982; Gallon, J.R., 1980
Synechococcus sp. ATCC 27145 PCC 6311	unicellular concentric thylakoids freshwater 26°C, 2000-3000 lux		Blumwald, E. et al., 1983
Synechococcus sp. ATCC 27264 PCC 7002 (Agmenellum quadruplicatum PR-6)	unicellular concentric thylakoids marine 39°C, 2000-3000 lux		Van Baalen, C., 1962
Gloeobacter violaceus ATCC 29082 PCC 7421	unicellular lacks thylakoids 22°C, < 500 lux		Rippka, R. et al., 1974

Table II

CELL COMPOSITION OF NOSTOC MUSCORUM GROWN UNDER WHITE (300-700nm) AND BLUE (400-500nm) LIGHT

Light Quality	Percent Dry Weight					
	Protein	Glycogen	Soluble Sugars	Lipid	Chlorophyll	Total
White	36	28	10	13	1.05	88
Blue	25	22	10	9	0.46	67

Samples taken after seven days of growth

waterbath at 28°C under 50uEs^{-1}m^{-2} blue or white light.

Light intensities were measured using a Li-Cor inc. Integrating quantum/radiometer/photometer Li-188B, with a Li-190SB quantum sensor.

Oxygen-broadening experiments:

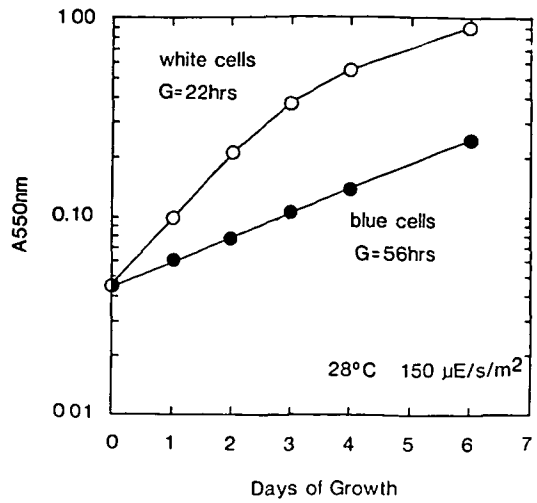
Dense suspensions (1-2 mg chl/ml) of the cyanobacterium Agmenellum quadruplicatum, in ASP-II medium, were introduced into 75 ul capillary tubes, together with 0.5 mM of the nitroxide spin-probe 3-hydroxy-2,2,5,5-tetramethyl 3-pyrrolin-1-oxyl (PCA-ol). The ESR signal was monitored by using a varian E109 EPR spectrometer. The sample was illuminated inside the cavity with an Oriel Universal Xenon Air Lamp.

Experimental Results

Representative strains of cyanobacteria are shown in Table I, along with their nitrogen fixing capabilities and growth conditions. The heterocystous filamentous strain Nostoc muscorum (Anabaena 7119) was chosen for the present study.

Growth of Nostoc muscorum under blue (400-500nm) and white (300-700nm) light at 150uE/s/m^2, is shown in Figure 1. Clearly the cells grow readily under the blue light,

Fig. 1) Growth of Nostoc muscorum under white (300-500 nm) and blue (400-500 nm) light, measured by absorbance changes at 550 nm. G=cell doubling time.



even though the cell doubling time (generation time, G) is increased by a factor of 2.5. Analysis of photosynthetic O_2 evolution and nitrogenase activity during growth under blue light reveal very little difference from those grown under white light when rates were calculated on a total protein basis (Figure 2 and 3, upper sections). Calculation of rates (particularly photosynthesis) on a total chlorophyll basis reveal an apparent increase in photosynthetic rate (Figure 2, lower section). However, this reflects a decrease in total cell chlorophyll, a chromatic adaption to blue light (see Table II). Analysis of cellular products after a seven day growth period under blue and white light reveals that little change occurs in soluble sugars, glycogen, or lipids, while total protein decreases only by 10% (Table II). An "unknown" component which does increase (see "Total" column in Table II), is probably cell wall material, and is under further characterization.

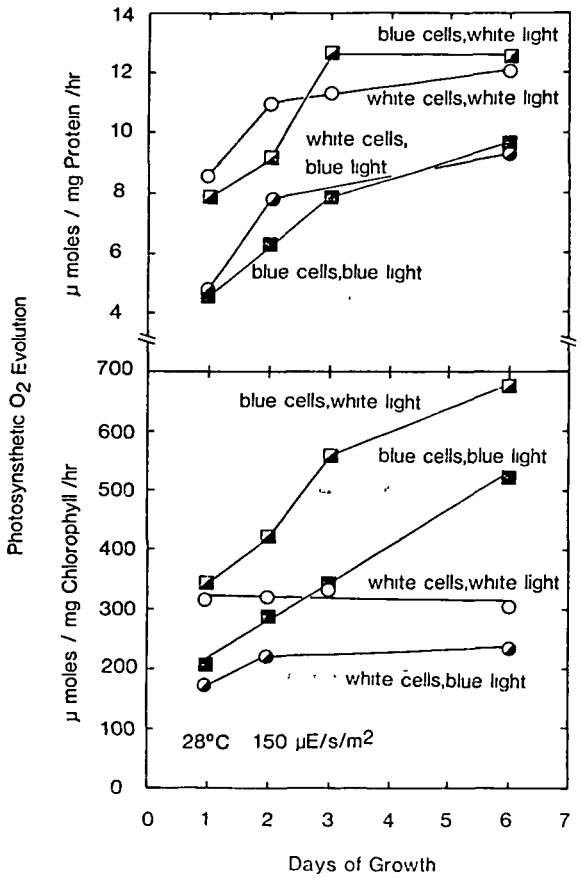


Fig. 2) Photosynthetic O_2 evolution of cells grown under white or blue light, based on total protein (upper secion) and chlorophyll (lower section) conditions as in Materials and Methods.

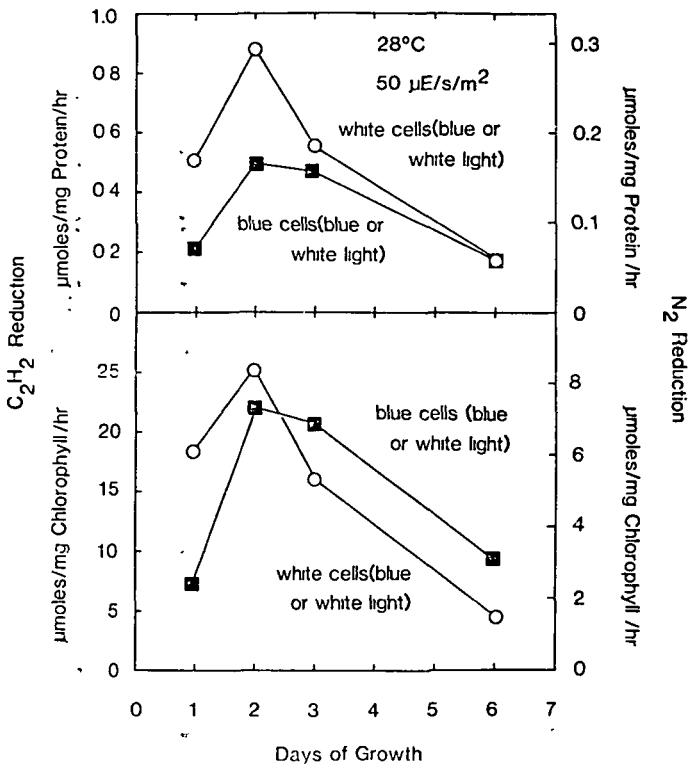


Fig. 3) Nitrogenase activity, as measured by acetylene reduction based on total protein (upper section) and chlorophyll (lower section) conditions as in Materials and Methods.

In conclusion, the nitrogen fixing strain Nostoc muscorum is capable of growth under light at the blue end of the visible spectrum. One must evaluate whether the energy saving gained by employing blue photons outweigh the slower growth rate (and hence biomass production). However, if a system employing a continuous culture (kept at a fixed point in the exponential growth phase, linear section of Figure 1) for air revitalization, etc., then a slower growth rate may be advantageous, requiring less addition of growth medium to maintain the constant cell

density.

Nitrogenase, the enzyme responsible for the biological fixation of atmospheric N_2, is extremely oxygen-sensitive[2]. One of the modes in which cyanobacteria "solved" the problem of N_2-fixation under aerobic conditions was the development of the heterocysts mentioned above. However, as with other nitrogenase containing microorganisms, the mechanisms by which these cells retain an oxygen-free internal environment is not completely understood.

We have recently developed an ESR technique which enables us to measure introcellular O_2 concentrations. We hope to utilize this method to gain a better understanding of the protection mechanisms against the toxic effects of O_2 to nitrogenase, and thereby of the ways the activity of this enzyme may be manipulated to the project's advantage.

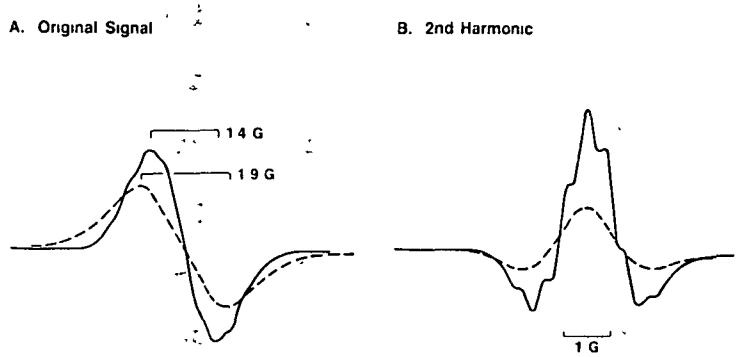


Fig. 4) The center ESR signal of PCA-ol (A) and the 2nd derivative (B), before (solid line) and after (dashed line) 1 min illumination of A. quadruplicatum suspension (1mg chl/ml). PCA-ol concentration was 0.5 mM.

The above technique is based upon the broadening effect dissolved O_2 has on the nitroxide spin probes ESR signal. An example of such an effect is presented in Figure 4. Photosynthetically-generated oxygen, in a suspension of the cyanobacterim Agmenellum quadruplicatum, causes a broadening of the signal by 0.5 G (Figure 4a). The effect is even more pronounced when a higher derivative of the spectrum is examined (Figure 4b). According to our calculations, this broadening is equivalent to O_2 concentration in the suspension of 2.5 mM. Furthermore, by quenching the extracellular signal, it is possible to examine only the probe inside the cells (Figure 5), and using the measured degree of broadening calculate intracellular O_2 concentrations. Studies are presently underway to compare O_2 levels in non-N_2-fixing and some N_2-fixing cyanobacterial cells, as well as to establish the O_2-sensitivity of various photosynthetic activities.

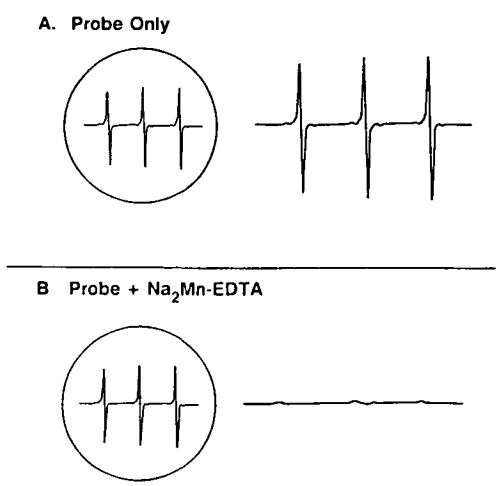


Fig. 5) A schematic presentation of the mode by which an internal spin-probe signal is differentiated from the external one.

Future Research

We propose to continue the investigation of the effects of selected wavelengths (and combinations of selected wavelengths) on biomass production and the biochemical functions of O_2 evolution and N_2 fixation.

In non-nitrogen fixing strains of cyanobacteria we have shown that changes in the environmental conditions, particularly increases in salinity, result in a marked increase in soluble sugars[6,7] and carbohydrate storage products[5]. We plan to investigate the partitioning of photosynthetically derived reductant between the various macromolecules (protein, lipid, carbohydrate) under such conditions to determine the ability to manipulate the relative levels of the various macromolucules by environmental changes.

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Carbon Dioxide Evolution Rate As a Method to Monitor and Control an Aerobic Biological Waste Treatement System

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INTRODUCTION

A long term manned space mission(ca.2 to 20 years) may require a regenerative life support system(RLSS). A closed ecological life support system(CELSS) or partially closed ecological life support system(PCELSS) may be suitable for this purpose. In a partial CELSS a significant portion of the food is expected to be grown on board. Waste material, principally consisting of inedible plant residues and human metabolic wastes, must be treated to yield CO_2 plus a mineral solution capable of supporting plant growth if acceptable system closure is to be attained.

Shuler(1979) has reviewed the advantages and disadvantages of physical- chemical and biological waste treatment systems within the context of CELSS. He suggested that generally a wet-oxidation process (physical- chemical) approach might be anticipated to be more space efficient but the form of outputs from biological processing may be more suitable to support plant growth (probably hydroponics).

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The objective of this investigation is to develop a mathematical model that can predict the performance of the biological waste treatment reactor which handles the complex chemically undefined feed. The reactor uses an undefined mixed culture of microorganisms. In this paper we will discuss how a simple carbon mass balance and carbon dioxide evolution information can be used as tools in modelling such a complex mixed culture and mixed substrate bioprocess. We will also present a mathematical model and compare the model predictions to experimental results both for steady-state and for transient conditions.

MODELLING APPROACH

Due to the complexity of the waste material to be biologically regenerated, the model is semi-empirical. Fig.1 illustrates our overall approach to modelling this system. The feed is considered to be composed of three major components: soluble and insolubles either non-lignocellulosics or lignocellulosics. Insoluble substrates must be hydrolyzed to soluble form before they can be utilized since we assume that the microbes will utilize only the soluble form of substrate. Therefore

OVERALL MODELLING APPROACH

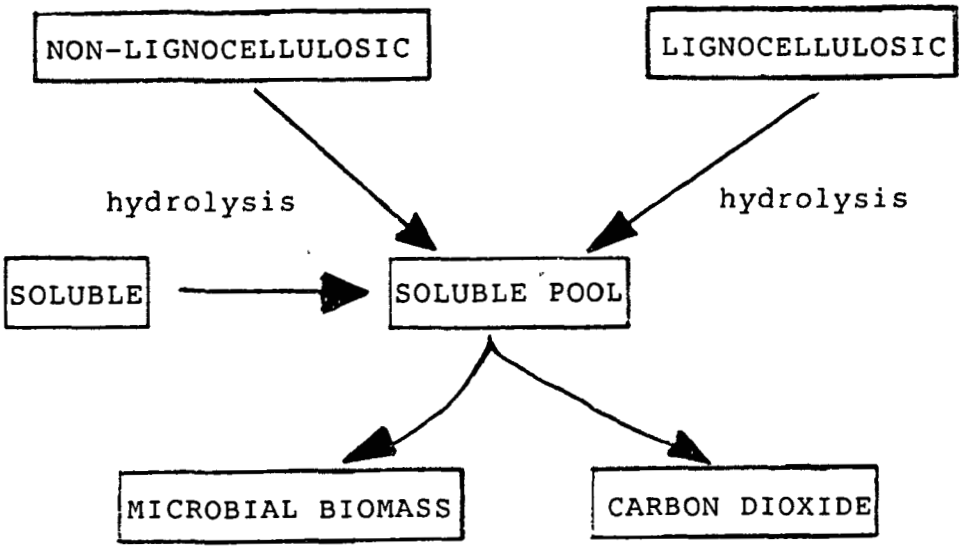


Figure 1. Overall modelling approach

the amount of insoluble non- lignocellulosic and lignocellulosic substrate utilized can be no more than than the amount hydrolyzed. Contributing to the total pool of soluble substrate is the soluble portion of waste in the feed and the hydrolysis products of insoluble non-lignocellulosics and lignocellulosics.

Therefore our model will be developed in two steps. The microbial growth model from only soluble substrate will be developed first, then we will develop the hydrolysis kinetic model of insolubles which will be coupled with the microbial growth model to describe the overall biodegradation of waste material.

CLEAN SYSTEM

To study the microbial growth kinetics from the soluble substrate, we have worked with a "clean system". In the "clean system" experiments we obtained a clarified feed by separating a soluble portion of the "real" waste by filtering the waste through a ultrafiltration unit with 10,000 M.W. cut off membrane. 5g/liter of glucose is added to the soluble portion to simulate the hydrolysis product of cellulose.

There are two major advantages in working with the "clean system". Firstly, it is easier to conduct experiments. The presence of insoluble lignocellulosics in the reactor causes many mechanical problems such as plugging of tubing, difficulties in maintaining homogeneous mixing , etc. Secondly, several different methods of estimating microbial cell mass can be compared. Estimating biomass with precision and accuracy is essential in studying microbial growth kinetics. However, it is very difficult to estimate cell biomass in a complex system where insoluble particles such as lignocellulosics are present in high concentration. One of the methods we have used successfully is a carbon balance and CO_2 evolution rate (CER) method. Using the "clean system" allowed us to test the validity of the carbon balance-CER method by comparing it to methods such as dry weight, and optical density. The advantage of the carbon balance-CER method is that since CER can be measured on-line, the cell biomass and growth rate can be obtained with speed and precision.

MATERIAL AND METHODS

MODEL WASTE

A model waste simulating that expected to be generated in a space ship with 20 crew members during a ten year mission has been formulated. The composition of model waste is given in Table 1. Cerophyl(Cerophyl Lab.,Inc., Kansas City, MO.) is dried and ground stems and leaves from young rye plants. It is used to mimic the inedible plant residues. Freeze- dried feces and urine were obtained from USDA. The food preparation wastes, freeze-dried and canned, were prepared by Prof.Karel of MIT to simulate the types of waste that might result from a predominantly vegetarian diet.

ORGANISMS

A mixed culture was originally obtained from soil and the Trumansburgh, NY activated sludge plant. The mixed culture is stored frozen(-20° C) prior to the inoculation of the experimental system.

TOTAL CARBON

Total carbon was analyzed using a total carbon analyzer Beckman Model 915A. Samples of feed, reactor and recycle

Table 1. Waste Model

Component			Quantity
1.	Urine		1.76 g
2.	Feces		1.24 g
3.	Cerophyl		11.03 g
4.	Food preparation waste[+]		1.10 g
5.	Wash water concentrate		0.47 g
		Wt. % of wash water concentrate solids	
	(a) Lactic Acid	9.0	
	(b) Urea	7.0	
	(c) Glucose	1.0	
	(d) Soap (Ivory)	30.0	
	(e) Purified Cellulose	35.0	
	(f) NaCl	9.0	
	(g) KCl	9.0	
6.	Synthetic spent nutrient		0.72 g
		Wt. % of Synthetic spent Nutrient Solids	
	(a) NaCl	65.4	
	(b) K_2HPO_4	5.0	
	(c) $CaSO_4 \cdot 2H_2O$	6.2	
	(d) $MgSO_4 \cdot 7H_2O$	3.8	
	(e) $FeSO_4 \cdot 7H_2O$	0.06	
	(f) H_3BO_3	0.02	
	(g) $ZnCl_2$	0.02	
	(h) $CuSO_4 \cdot 5H_2O$	0.01	
	(i) Cinnamic Acid	19.5	
7.	Distilled-deionized H_2O		1,000 ml
	Total Solids - 16.3 g/l; pH = 5.6		

[+]Supplied by Prof. Karel at MIT

were hydrolyzed by concentrated H_2SO_4 (98%) immediately after sampling. A 10ml portion of the sample was mixed with 10ml of concentrated H_2SO_4 in a glass vial at room temperature. The vial was shaken gently several times. The acid hydrolyzed samples were stored at room temperature until they were analyzed. A soluble portion was obtained by centrifuging the sample then filtering the supernatant through 0.45 um membrane filter. The soluble sample was then stored frozen(- 20° C) prior to analysis.

CO2 MEASUREMENTS

Carbon dioxide concentration in the effluent gas was measured by using a Gas Chromatograph(Aerograph Series 2700, Varian Associates,Inc., Palo Alto, CA), with a thermal conductivity detector (F and M Scientific 700 Laboratory Chromatograph, Hewlett- Packard, Inc.). A Porapak Q column was used.

DISSOLVED OXYGEN (D.O.)

Dissolved oxygen concentration of the reactor was measured with D.O. probe (Leeds and Northrup Electronics, PA) and monitored intermittenly.

OPTICAL DENSITY (O.D.)

Optical density was measured by Bausch and Lomb Spectronic 20 spectrophotometer at 600 nm.

VIABLE CELL COUNT (V.C.C.)

Viable cell count was determined by spread plate method on CGY medium(Unz and Dondero,1967) after mild sonication of the 50ml sample in a 100ml beaker to disperse microbial flocs. A Sonic Dismembrator Model 300 was used with a power setting of 25. The sample beaker was immersed in a water bath at room temperature during the sonication to cool down the sample. The plates were incubated at 30° C for 3 days.

CELLULOSE,HEMICELLULOSE AND LIGNIN

Cellulose, hemicellulose, and lignin were determined by acid- detergent method (Goering and Van Soest,1970). The analysis was performed by Dr.J.Robertson of the department of Animal Science, Cornell University.

CHEMOSTAT EXPERIMENTS

Clean System

Continuous experiments with "clean" feed (soluble portion

of the model waste with 5g/l of glucose added) were conducted using a 2 liter Multi-gen (New Brunswick Sci. Co., New Brunswick, NJ) with a working volume of 1.2 liter. The reactor was controlled for temperature and pH of 7.0 by adding 2N H_2SO_4 as required (via NBS pH controller).

Real System

Continuous experiments with "real" model waste(which includes insoluble fractions) were conducted in a 7.5 liter NBS Model 19 Fermenter operating with 4 liter working volume. The experimental system is shown in Fig 2. pH was maintained at 7.2 by pH controllers (NBS). 2N H2SO4 and 2N NaOH was used for acid and base addition. The temperature was also controlled and dissolved oxygen(D.O.) was monitored intermittenly using a Leeds and Northrup D.O. probe on a slip stream. Aeration was achieved by sparging air directly underneath the bottom impeller. Three impellers (turbine flat blade type) were used for aeration and agitation. Air flow rate was maintained above 1.5 vvm and agitation was higher than or equal to 400 rpm. Air flow rate and agitation were varied to maintain D.O. above 2 mg/liter. Excessive mixing is undesirable since foaming may result.

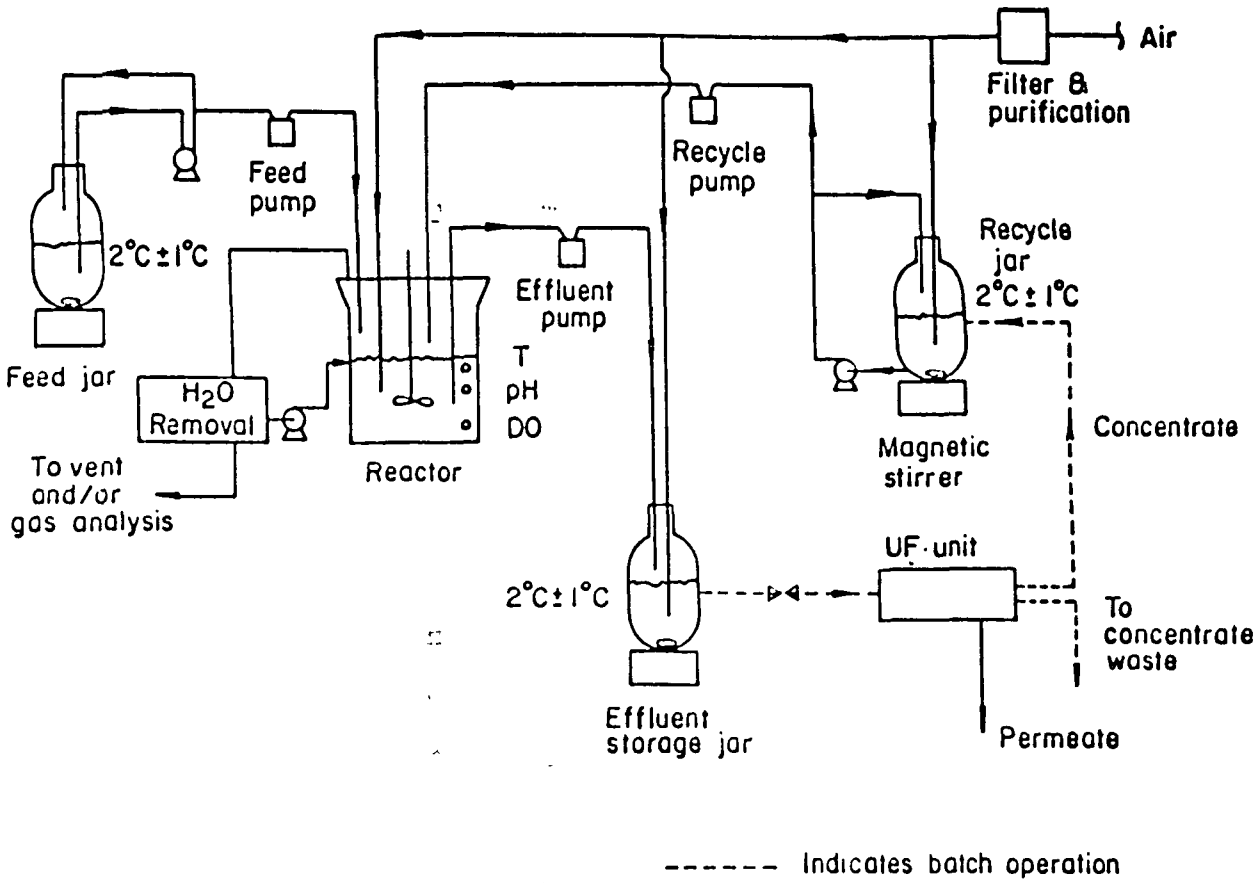


Figure 2. Flow diagram of "real" experimental system

RESULTS AND DISCUSSION

Experimental data from chemostat experiments for the "clean system" are listed on the Table 2. Dilution rate was the independent parameter. Data are from steady-state operation.

TABLE 2: Experimental Results for "Clean System" at 25°C

Θ_H(hr)	S(mg/lℓ)	C.E.R. (mg/ℓ-hr)	Total Carbon in Reactor (mg/ℓ)	Biomass (mg/ℓ)
7.5	400	292	1968	1568
9.7	295	224	1936	1641
11.2	279	191	1636	1357
16.0	407	132	1932	1525
40.8	203	61	1728	1525

Notes:

1. All data expressed as total carbon concentration.
2. Substrate concentration, S, included recalcitrant portion of 200 mg/ℓ.
3. C.E.R. = CO_2 evolution rate.
4. Feed concentration, S_o, was 4200 ppm.
5. Biomass, X, is estimated by simple carbon mass balance (see eq. 1).

CARBON BALANCE

Mass balance of the system is necessary information for a CELSS. Traditional characterization of substrate level as BOD or COD does not provide material balance information. To obtain carbon mass balance, all the samples were analyzed for total carbon. This procedure allows us to estimate the microbial biomass concentration from a simple carbon balance. For the "clean system" :

$$Rc = Xc + Sc \qquad (1)$$

where Rc = carbon content of reactor sample(mg/l)
Xc = microbial biomass as carbon (mg/l)
Sc = soluble substrate concentration as carbon(mg/l)

The microbial biomass, Xc, may be estimated by difference using eq.1, since Rc and Sc can be measured experimentally. Fig.3 shows the microbial biomass estimated by the carbon balance method compared to the experimental results of dry weights(D.W.) measurements and O.D. measurements for the "clean system". The average carbon content of microbial biomass(measured by by D.W.) is calculated to be 38 %. This is lower than the value of 46.2 % reported by Erickson and his co-workers(1979). However, the graph shows that the results of all three different method are consistent with

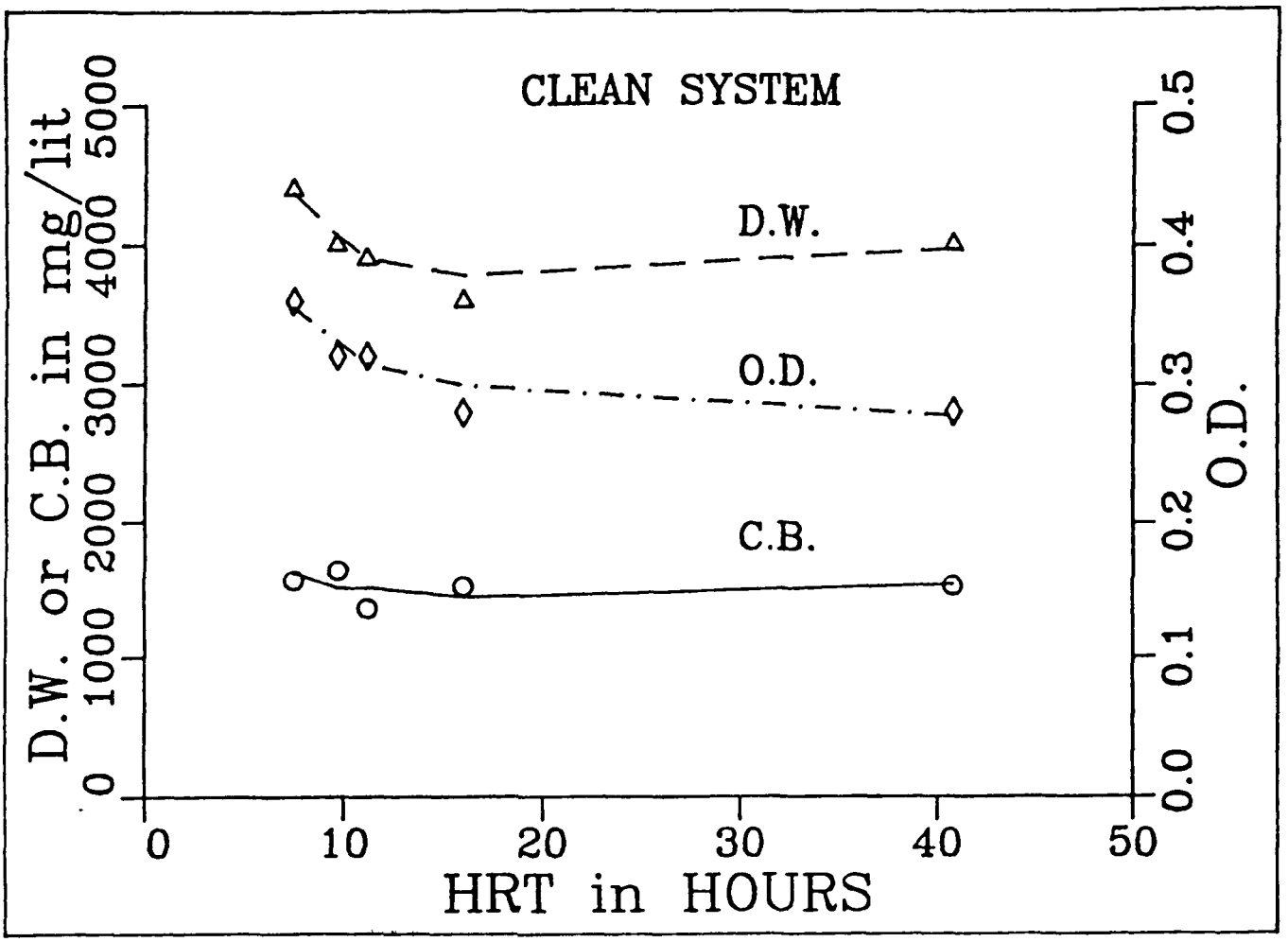


Figure 3. Comparison of different biomass estimation methods: Dry Weight(D.W.), Optical Density(O.D.), and Carbon Balance(C.B.)

each other. The graph also shows that the biomass increases as the dilution rate (D) increases. This may be explained by the assumption that the maintenance energy requirement will be proportionally less as the growth rate increases(Pirt,1965). The carbon balance measurements can be checked for the consistency using carbon dioxide evolution rate(CER) measurements (see eq.5 and Fig.4).

IMPORTANCE OF CER

The carbonaceous substrate may be incorporated into microbial biomass, evolved as respiration by products, or incorporated into extracellular products. The overall balance equation of aerobic microbial reaction may be written as (Erickson et al.,1979)

$$CHmOl+aNH3+bO2 = ycCHpOnNg+zCHrOsNt+cH2O+dCO2 \quad (2)$$

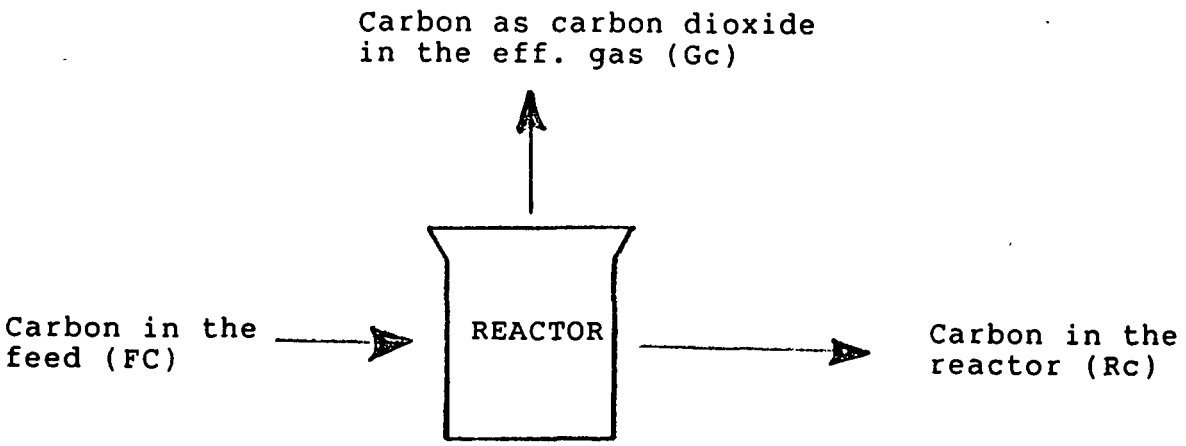
where CHmOl = elemental composition of the organic substrate
CHpOnNg = elemental composition of the cell biomass
CHrOsNt = elemental composition of any extracellular products

If we consider the carbon balance

$$yc + z + d = 1.0 \quad (3)$$

and when no extracellular products are formed (z = 0)

$$yc + d = 1.0 \quad (4)$$



$$Gc = (CER)(HRT) = Fc - Rc$$

Figure 4. Carbon Balance

Eq.4 shows that the amount of carbon in the effluent gas as CO_2 in our "clean system" experiments should be equal to the differential amount of carbon between the feed and the reactor (see Fig.4). The carbon dioxide evolution rate (CER) is obtained by

CER(mg/l-hr) = Carbon in CO_2/Residence time(HRT)

Therefore CER may be used to check the consistency of the experimental measurements since we may write

$$(CER)(HRT) = Fc - Rc \qquad (5)$$

where Fc = feed concentration as carbon (mg/l)

CER can also be used to estimate the microbial biomass directly once the relationship between the CER and biomass is determined for a given system and a set of environmental conditions. When the substrate acts as both a carbon and energy source for an organism accurate estimation of the part of the carbon source which is assimilated and that part which is dissimilated to provide energy is difficult. However, adequate estimation often can be made with the following method(Pirt,1965).

For an aerobic system we assume that the oxygen is used exclusively as the final electron acceptor in energy yielding process(Hernandez and Johnson,1967) and that the amount of carbon in CO_2 is equivalent to the amount of carbon substrate dissimilated to provide energy. Pirt(1965) has suggested that the substrate used to produce biomass may be composed of two fractions;

$$\begin{array}{l} \text{total substrate} \\ \text{utilized } (\Delta St) \end{array} = \begin{array}{l} \text{substrate utilized to} \\ \text{produce cell carbon } (\Delta Sc) \\ \qquad + \\ \text{substrate utilized to} \\ \text{provide energy } (\Delta Se) \end{array} \qquad (6)$$

If we divide the substrate balance by ΔX, that is the amount of biomass produced, we obtain

$$\Delta t / \Delta X = \Delta Sc / \Delta X + \Delta Se / \Delta X \qquad (7)$$

which can be written as

$$1/Y = 1/Yc + 1/Ye \qquad (8)$$

where Y = overall yield ($\Delta X / \Delta St$)
Yc = biomass yield ($\Delta X / \Delta Sc$)
Ye = energy yield ($\Delta X / \Delta Se$)

for a carbon balance case

$$Yc = 1$$

Then we obtain

$$1/Y = 1 + 1/Ye \qquad (9)$$

As discussed earlier

ΔSe = carbon as CO_2 in effluent gas for aerobic system

$$CER = \Delta Se / HRT = (\Delta Se)(D) \quad (10)$$

$$Ye = \Delta X / \Delta Se = (D)(X) / CER \quad (11)$$

Since for chemostat $\Delta X = (X - Xo) = X$, combining eq.11 with eq.9 we obtain

$$1/Y = 1 + CER / (D)(X) \quad (12)$$

It has been postulated (Pirt,1965) that microbes require energy both for growth and for maintenance purposes. Therefore the total substrate dissimilated to provide energy , ΔSe , is assumed to be composed of two fractions: a portion for growth (ΔSeg) and the other portion for maintenance (ΔSem). This can be written as

$$Ye = \Delta X / \Delta Se = \Delta X / (\Delta Seg + \Delta Sem) \quad (13)$$

when the maintenance energy requirement is zero, that is M Sem = 0, we have the 'true' growth yield given by

$$Yeg = \Delta X / \Delta Se \quad (14)$$

combining eq.11,13, and 14 we obtain

$$1/Ye = CER / DX = 1/Yeg + \Delta Sem/X \quad (15)$$

And when we introduce the maintenanace energy coefficient,m(1/ hr), we obtain (Pirt,1965)

$$1/Ye = 1/Yeg + m/ \mu = CER/DX \quad (16)$$

and the eq. 12 can be written as

$$1/Y = (1 + 1/Yeg) + m/\mu \quad (17)$$

since for a chemostat $\mu = D$, we obtain

$$1/Ye = 1/Yeg + m/D = CER/DX \quad (18)$$

and

$$1/Y = (1 + 1/Yeg) + m/D \quad (19)$$

The utility of the above two equations is that we can estimate biomass from the CER measurements for a given system, since the overall yield and the yield based on the energy provided (or CO_2 evolved) are expressed as a function of CER and D. When eq.18 and eq.19 are plotted (CER/DX vs 1/D and 1/Y vs 1/D) these two equations should both have the same slopes, m, and their interceptors should have a difference of 1.0 . Fig.5 shows such a plot for the "clean system" at 25^{o} C. This graph demonstrates how CER measurements may be used to check the consistency of the carbon balance experimental results.

We have thus far discussd how CER can be used to check the experimental results of carbon balance method to estimate biomass and how it can be also used to estimate biomass directly once we have obtained Pirt's relationship expressed by eq.18 and eq.19 for a given

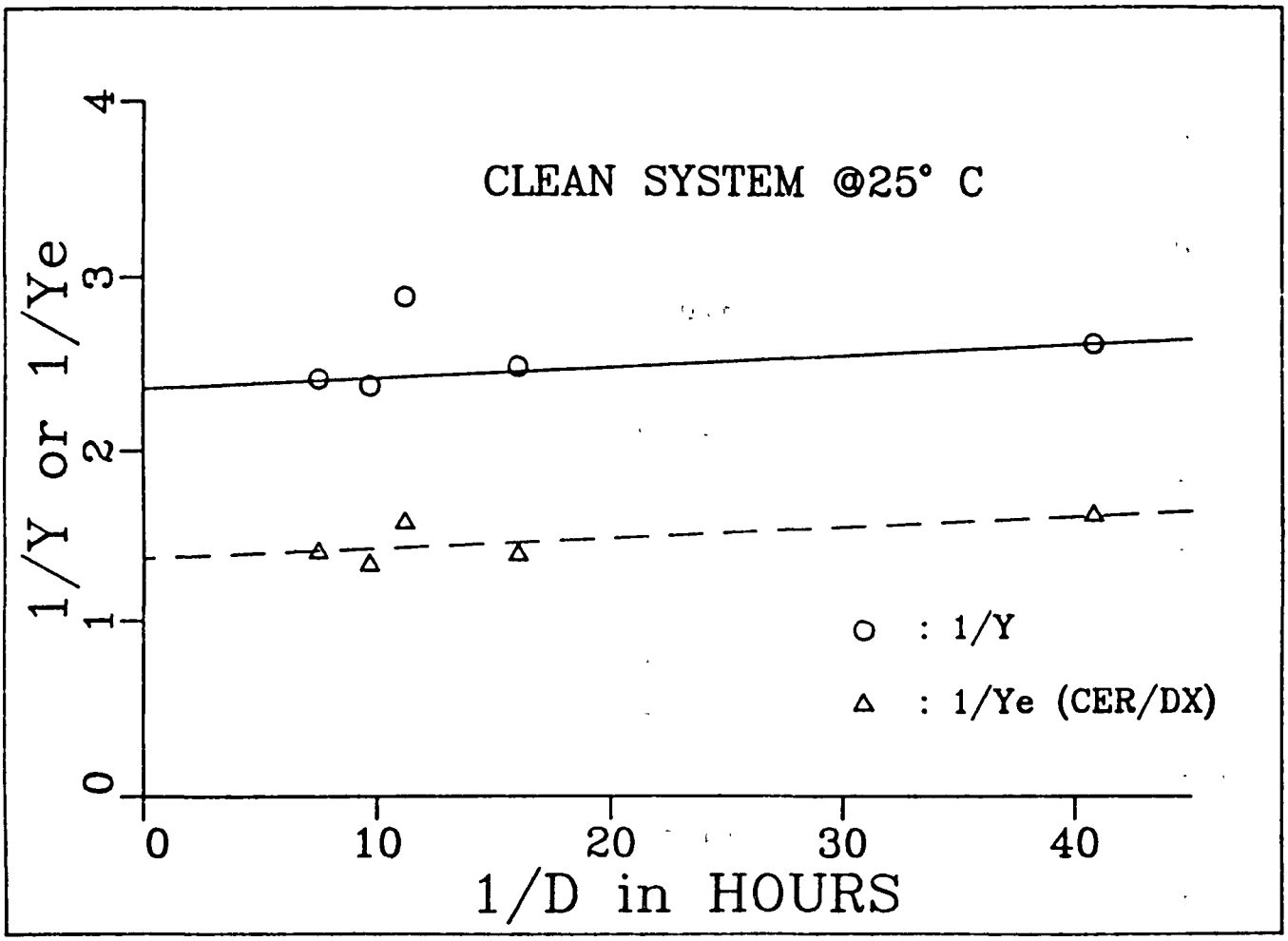


Figure 5. Graph of 1/Y and 1/Ye vs 1/D

experimental system. The advantage of using CER - carbon balance approach is that CER can be measured on-line with speed and precision. Thus biomass, and growth rates may be estimated almost instantaneously.

RELIABILITY OF CER MEASUREMENTS

We have seen that CER can give you a very useful information, and it can be measured on line. It is an excellent candidate for use as a process control parameter. Several investigators have suggested using CER as a process control parameter(Bravard et al., 1979; Mou and Cooney, 1983; Heijnen and Roels, 1979). Some of the criteria for an effective process control parameter is that it must be reliable,reproducible and sensitive to the changes in environmental conditions. Fig.6 shows the experimental results of CER measurements obtained at different hydraulic residence time(HRT) for four different experimental systems. They all display similar response as a function of HRT. The data for the pure culture of E.Coli grown on the glucose limiting media at 37^{o}C is from the work of Domach(1983). CER for this E.Coli system was lower than other systems since the influent substrate concentration, So, was only 1,000 ppm while the So's for the "clean system" were 4,200 ppm.

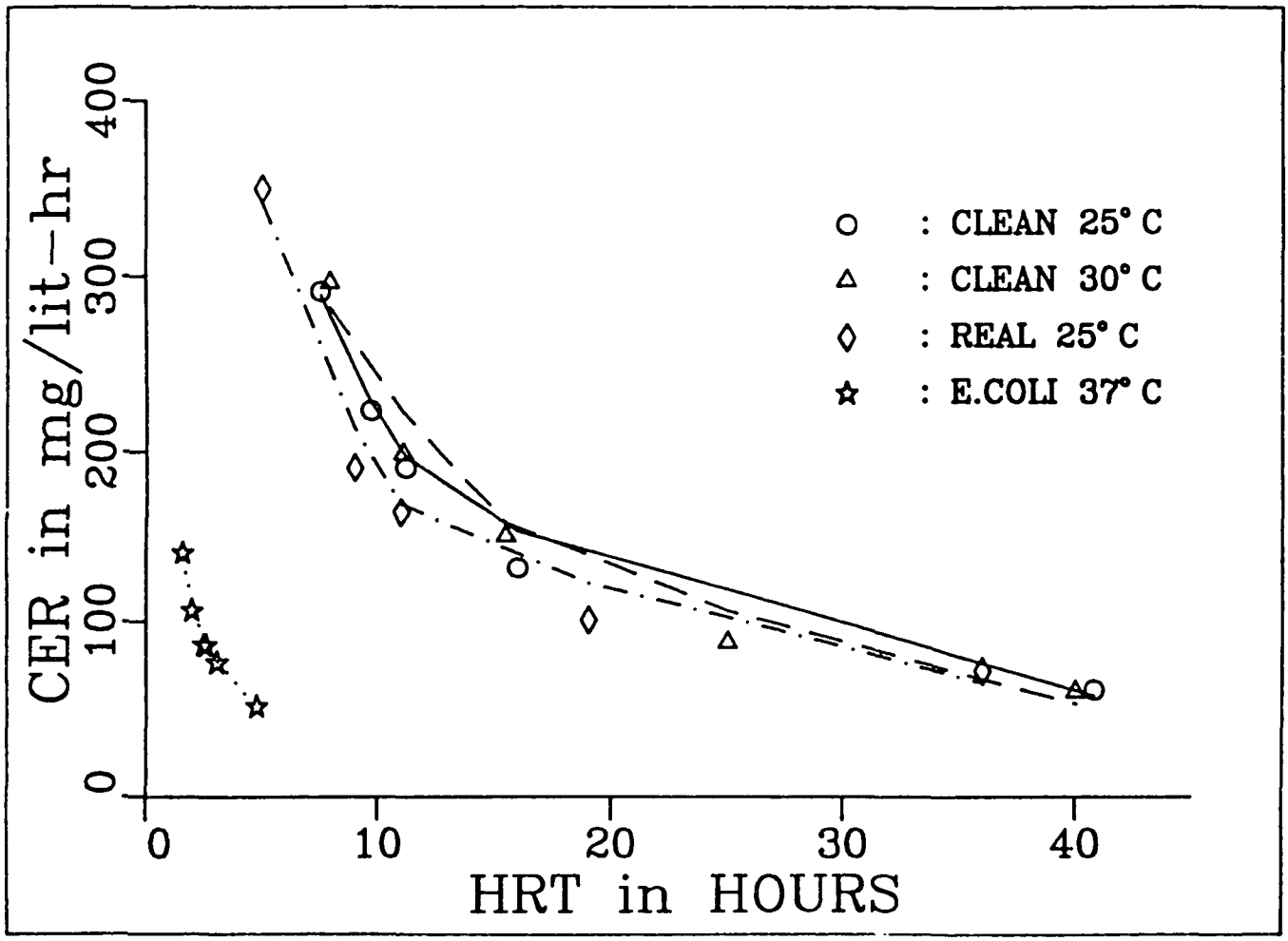


Figure 6. CER measurements vs HRT for four different systems

"So" for the "real system" varied with different HRT's since the amount of lignocellulosic substrate hydrolyzed added to the pool of soluble substrate and varied with HRT. The hydrolysis kinetic model of lignocellulosic substrate to soluble substrate will be presented in a future paper.

MODELLING OF MICROBIAL GROWTH KINETICS

The detailed description and theoretical justification of the model we have used will be discussed in a future paper. For now, we will briefly outline the basic assumptions and mathematical expressions of the model which was originally suggested by Andrews and Tien (1977). However they did not not test the model with chemostat experiments. We have extended the model to predict not only the steady state but also for transient responses, and the experimental verification of the model is discussed in this paper.

We assume that 1) the mixed culture may be represented as a single population of microbes which has the average properties of the mixed culture; 2) the biotic phase is assumed to have two distinctively

separate compartments; protoplasm which is capable of growth and other metabolic reactions and the stored substrate portion within the cell which is not yet oxidized; and 3) the microbial growth rate is directly proportional to the amount of stored substrate the cell has. The maximum growth rate occurs when the cell has the maximum amount of stored substrate within the cell. Thus:

$$\mu / \mu \text{ max} = \text{Stored substrate} / \text{Max. stored substrate}$$

$$= (Z)(P) / (Z)\text{max}(P) \qquad (20)$$

where μ = specific growth rate (1/hr)
μ max = max. specific growth rate (1/hr)
Z = ratio of stored substrate to the protoplasm concentration
Zmax = max. ratio of stored substrate to the protoplasm concentration

A furthur assumption is that the byproduct of metabolic energy producing reactions is CO_2 and the energy requirement is composed of two portions; growth associated and maintenance associated portions.

The underlying idea of these assumptions is that the microbial growth has two rate limiting reactions. The first one is the uptake rate of soluble substrate into the cell mass. The second one is the growth step where this stored substrate is used to produce additional

protoplasm and CO_2 is generated as a byproduct. Fig.7 illustrates these steps graphically.

The rate expressions for these steps were proposed by Andrews and Tien (1977). The uptake rate of substrate may be written as:

$$Rs = (Ku)(S)(P)(1 - Z/Zmax) \quad (21)$$

where Rs = substrate uptake rate (mg/l-hr)
Ku = kinetic constant (l/mg-hr)
S = substrate concentration (mg/l)
P = protoplasm concentration (mg/l)

The growth rate may be written as:

$$Rg = (Kg)(Z)(P) \quad (22)$$

where Rg = growth rate (mg/l-hr)
Kg = kinetic constant (1/hr)

The rate of metabolic reaction by product formation may be written as:

$$CER = (Kr)(Rg)+(Kb)(P) = (Kr)(Kg)(Z)(P)+(Kb)(P) \quad (23)$$

where Kr = growth associated kinetic constant(mg/mg)
Kb = maintenance associated kinetic constant (1/hr)

Using these rate equations we may write material balance equations for substrate concentration, protoplasm concentration, and storage concentration for chemostat:

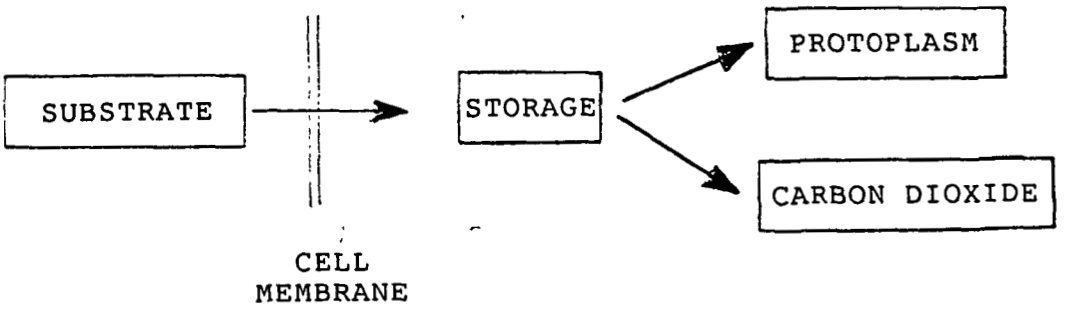


Figure 7. Graphic representation of substrate uptake step and stored substrate utilization step.

$$dS/dt = (D)(So - S) - Rs \quad (24)$$

$$dP/dt = -(D)(P) + Rg \quad (25)$$

$$d(Z)(P)/dt = -(D)(Z)(P) + Rs - Rg - CER \quad (26)$$

Substituting eq.21,22, and 23 into eq.24,25, and 26 we obtain:

$$dS/dt = (D)(So-S) - ((Ku)(S)(P)(1 - Z/Zmax)) \quad (27)$$

$$dP/dt = -(D)(P) - (Kg)(P)(Z) \quad (28)$$

$$dZ/dt = (Ku)(S)(1 - Z/Zmax) - (Z)(Kg) - (Z)(Z)(Kg) - (Kg)(Kr)(Z) - (Kb) \quad (29)$$

To use these equations we need to determine the kinetic parameters Ku,Kg,Kr,and Kb. These can be obtained from steady state chemostat data. Eq.27,28 and 29 can be solved using 4th order Runge-Kutta method.

Fig.8,9 and 10 show the steady-state experimental data compared with the model predictions for the "clean system" at 25^{O} C and 30^{O} C. The overall yield, CER, and 1/D vs 1/S were predicted very well. Biomass is represented as X = (P)(1 + Z) in our model. Perhaps the best way to test the plausibility of a model is to see how well the transient responses can be predicted. Fig.11 shows the experimental CER measurements as a function of time when we increased the dilution rate from 0.0245/hr to 0.1030/hr. It can be seen that CER

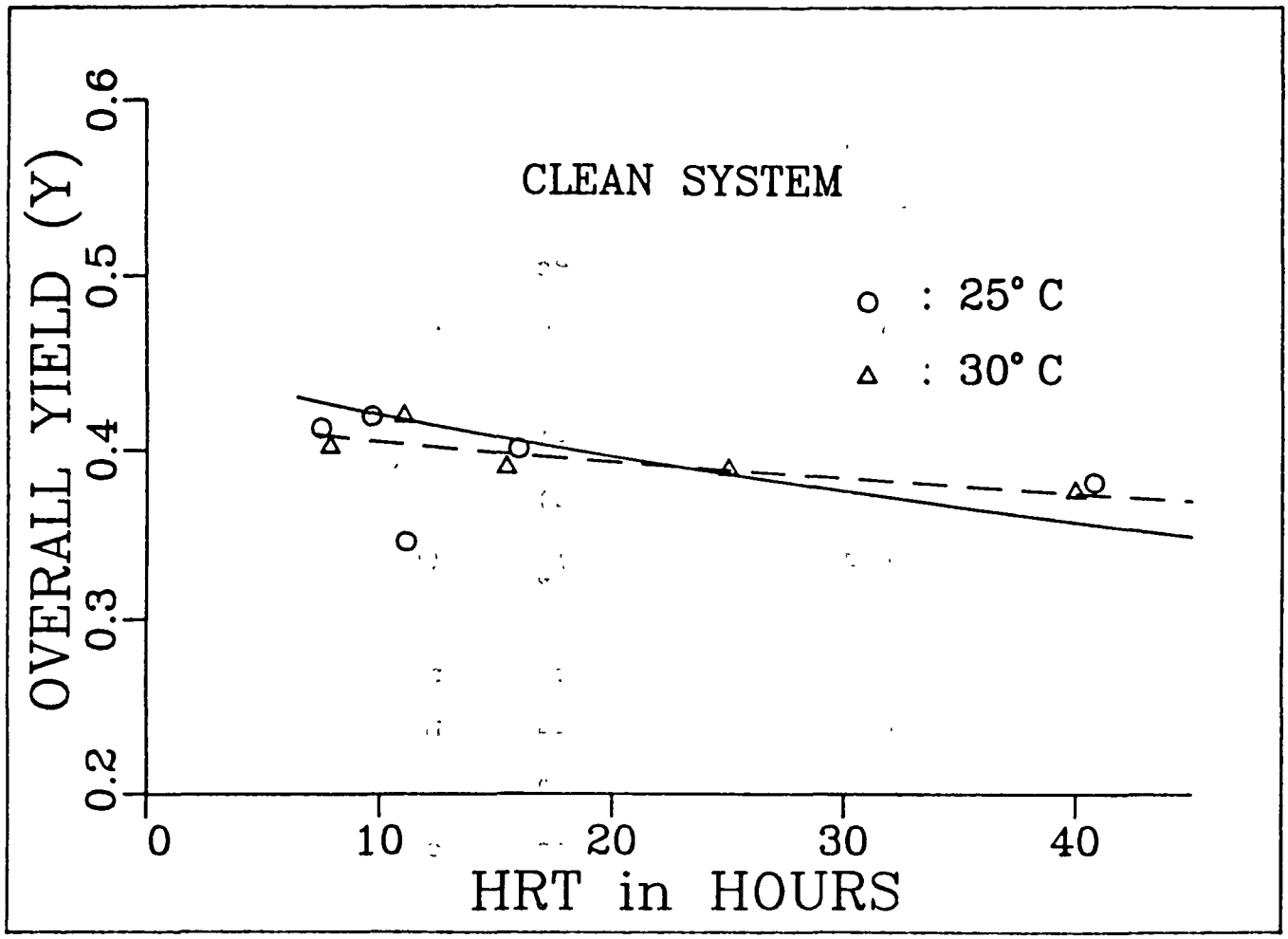


Figure 8. Comparison of experimental data and model prediction for overall yield(Y) for "clean system". Model predictions are represented by solid line for 25° C,and dashed line for 30° C.

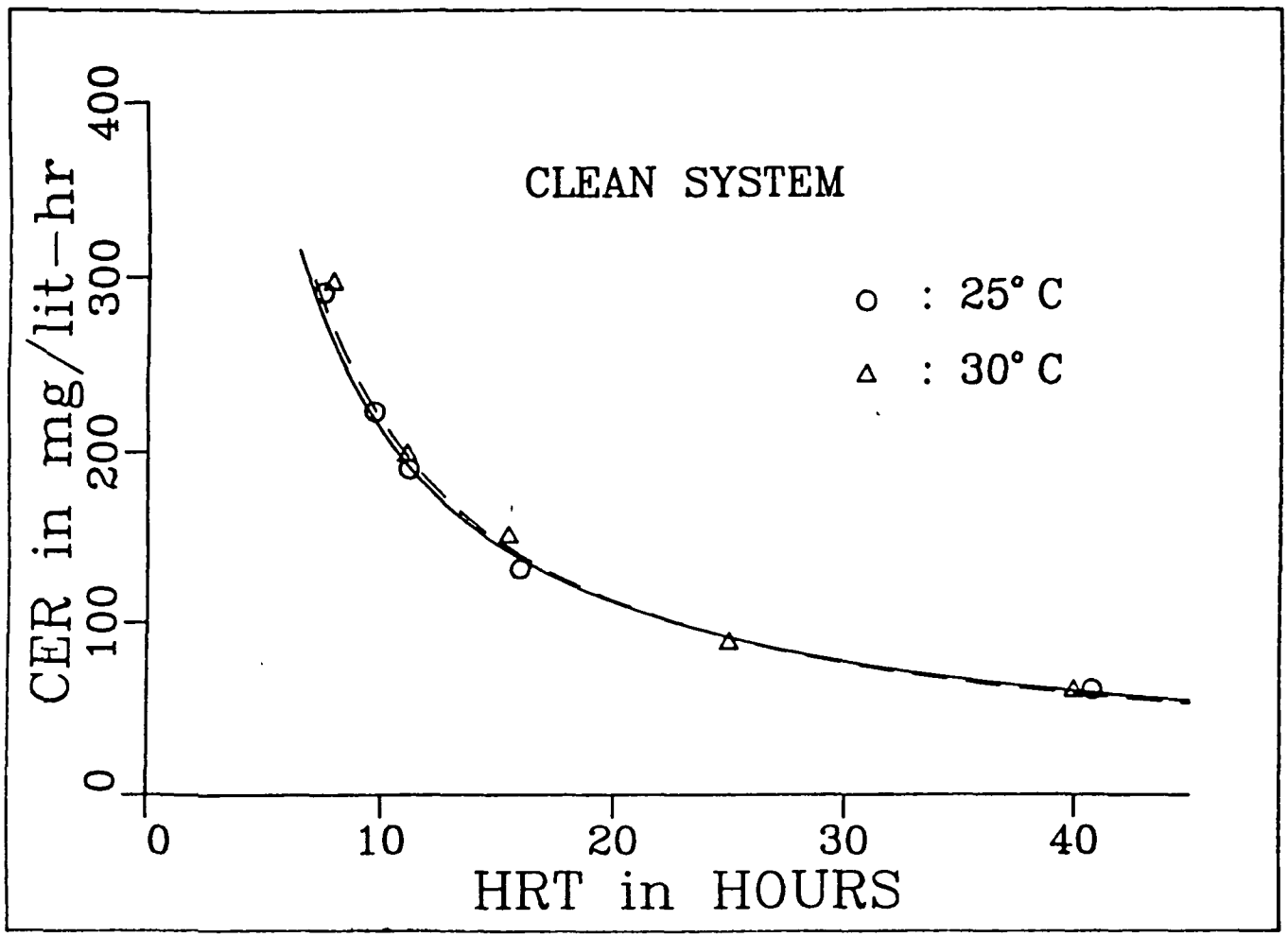


Figure 9. Comparison of experimental data and model predictions for CER for "clean system".Model predictions are represented by solid line for 25° C,and dashed line for 30° C.

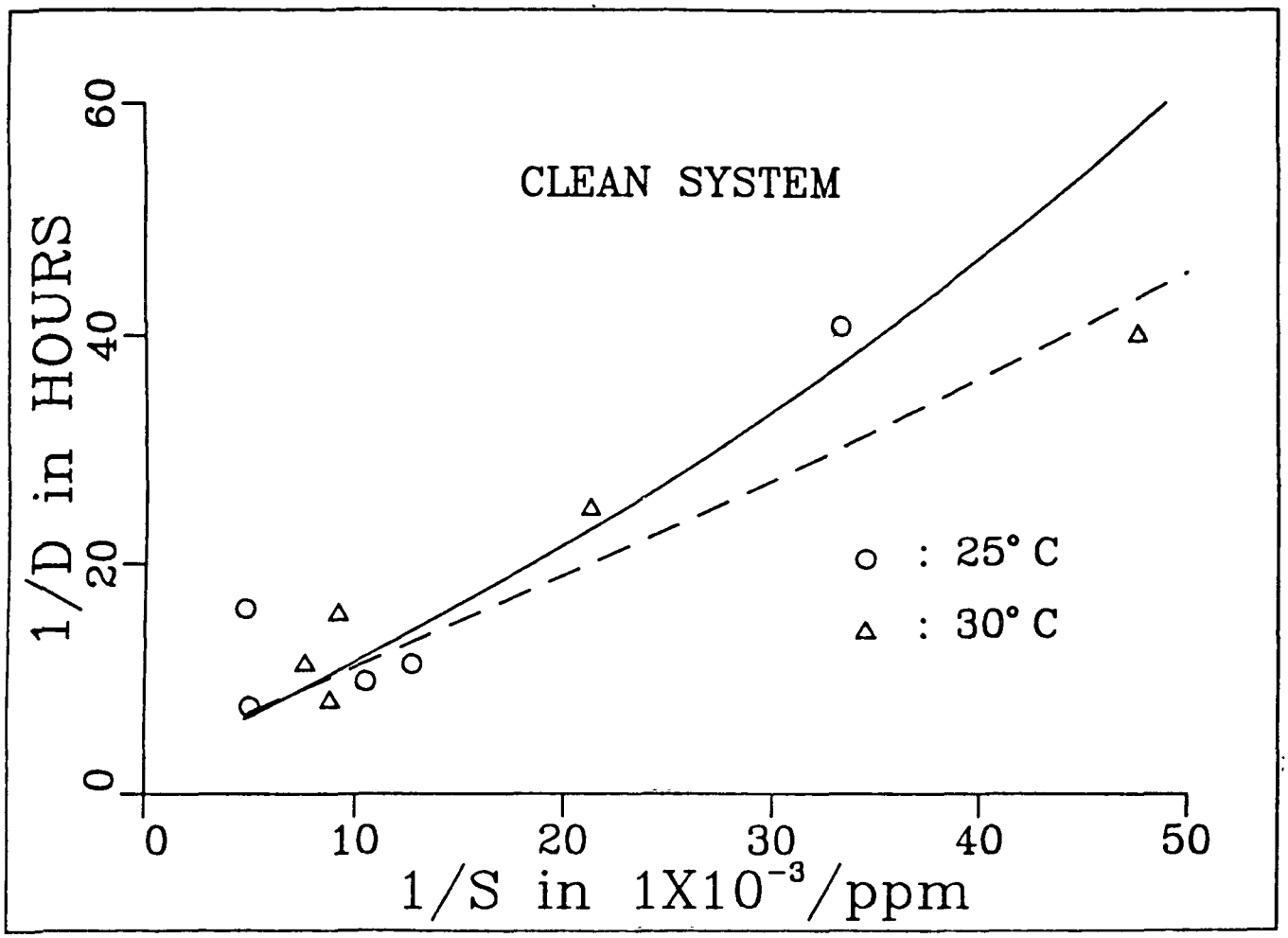


Figure 10. Comparison of experimental data and model predictions for 1/D VS 1/S for "clean system". Model predictions are represented by solid line for 25° C, and dashed for 30° C.

responded almost instantaneously after the increase of the dilution rate. This CER data is reproducible which furthur demonstrates the sensitivity and the reliability of CER measurements. The model predicts the transient response in CER remarkably well. The broken line in Fig.11 is the model prediction using Powell's model(Powell,1969). Powell's model is basically a Monod model modified to account for the time delay in cellular response to changes in growth rates. It is evident that our model predicts the transient response better. Fig.12 shows experimental CER data compared to the model prediction as we decreased the dilution rate from 0.127/hr to 0.025/hr. The model prediction is again excellent. Perhaps the most important results of this investigation is that the model we used can predict the transient response very well without the use of adjustable parameters.

The modelling of "real system" needs to incorporate the hydrolysis kinetic model for lignocellulosic substrate. The hydrolysate production rate equation from the hydrolysis model will be added to eq.27 as an added soluble substrate generation term. The hydrolysis model of lignocellulosic substrate and the overall model have

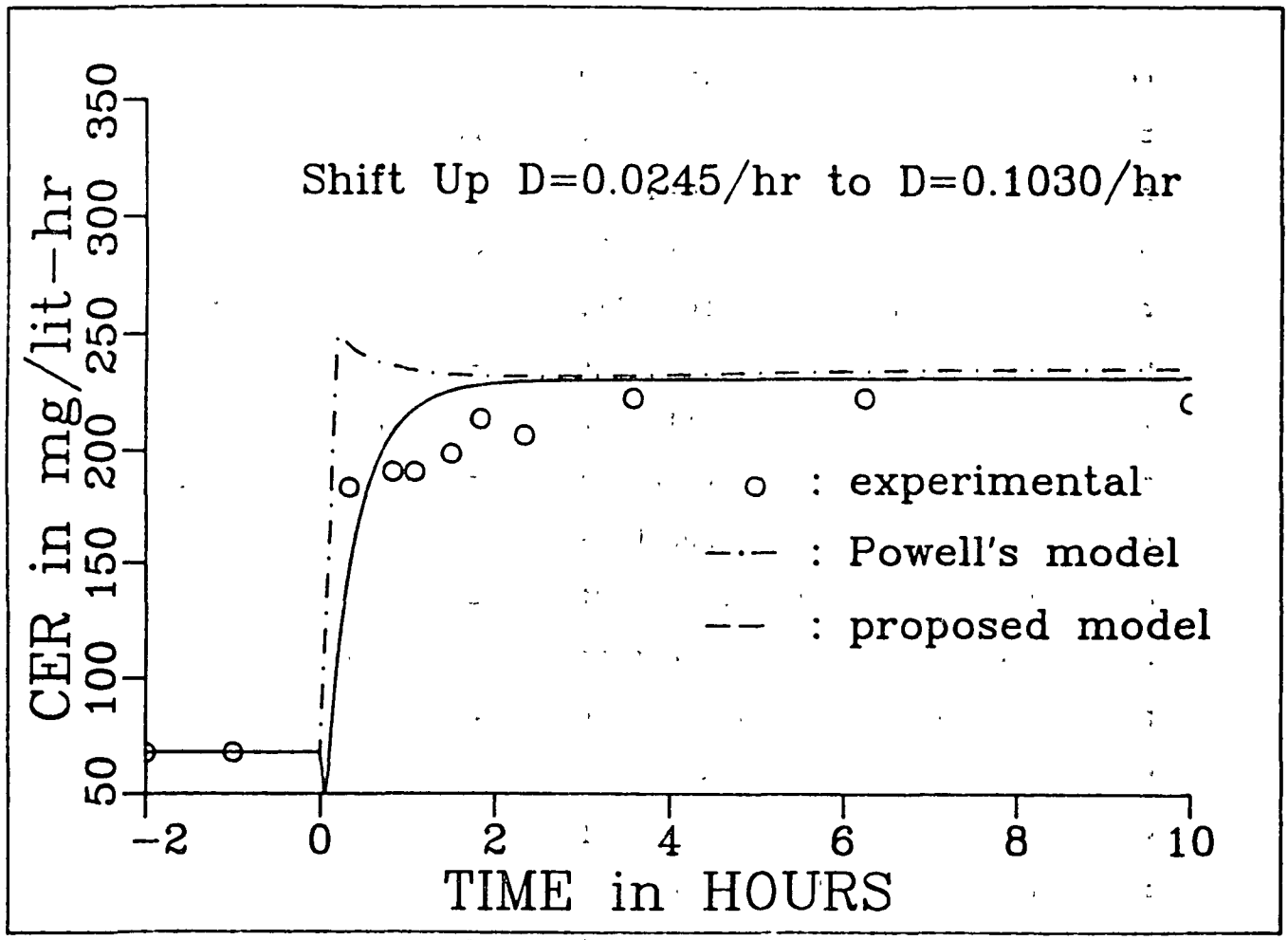


Figure 11. Comparison of CER data and model prediction of shift-up experiments.

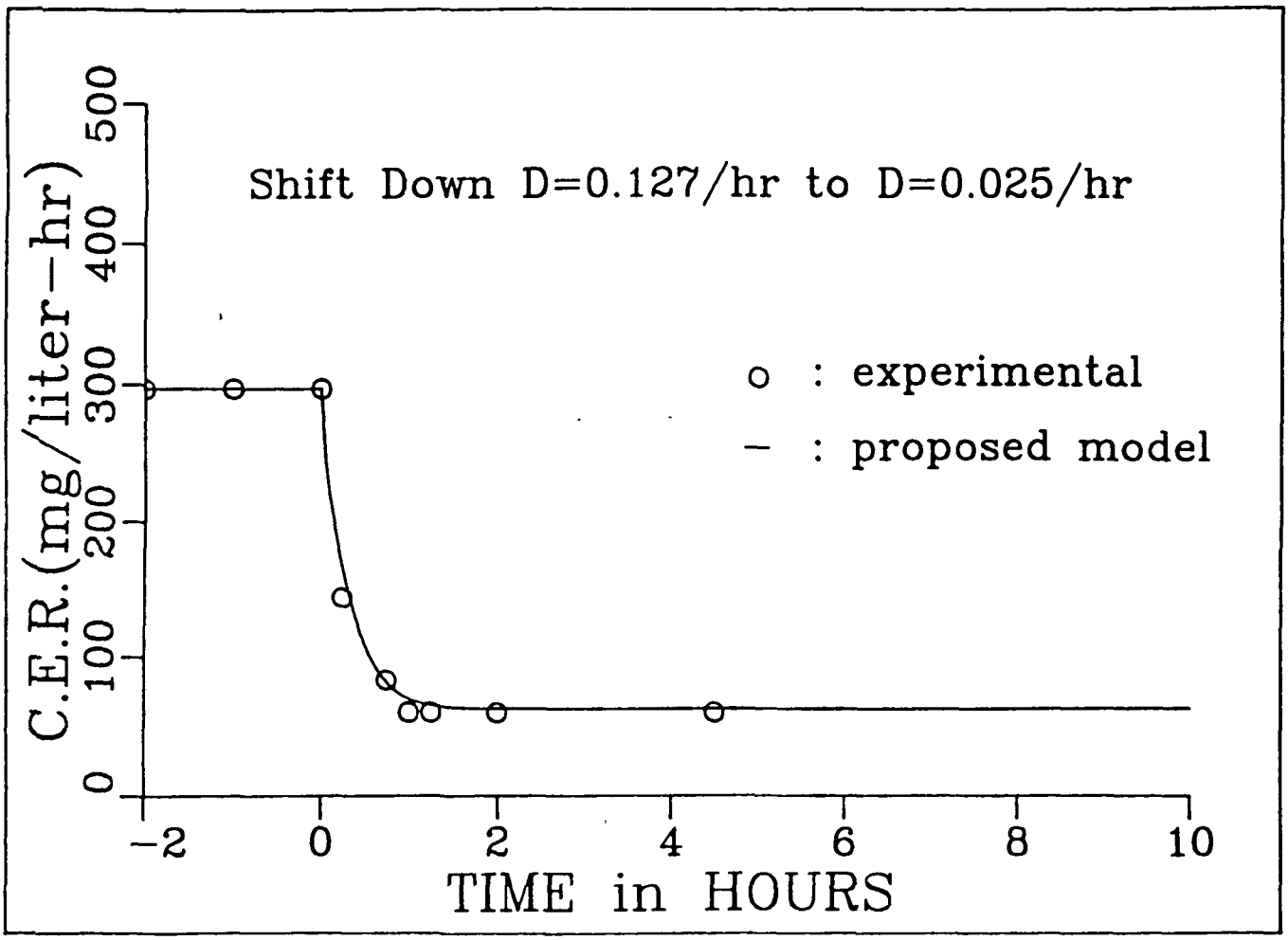


Figure 12. Comparison of CER data and model prediction of shift-down experiments.

been developed and are being verified experimentally.

SUMMARY

An experimental system was developed to study the microbial growth kinetic of an undefined mixed culture in an aerobic biological waste treatment process. The experimental results were used to develop a mathematical model that can predict the performance of a bioreactor. The bioreactor will be used to regeneratively treat waste material which is expected to be generated during a long term manned space mission. Since the presence of insoluble particles in the chemically undefined complex media made estimating biomass very difficult in the "real system", a "clean system" was devised to study the microbial growth from the soluble substrate.

A carbon mass balance was used to estimate microbial biomass. The CO_2 evolution rate(CER) was used to check the consistency of the carbon balance experimental results. CER can also be used to estimate biomass directly once Pirt's relationship with maintenance energy coefficients is known for a given system. The advantage of using CER is in its speed and precision. CER has been

demonstrated to be a reliable, reproducible, and sensitive indicator of biochemical state of a microbial system. Since CER can be monitored continuously on-line it can be used to control the biochemical reactor.

A mathematical model of microbial growth originally suggested by Andrews and Tien(1977) has been extended to study the chemostat processes. The model predictions have been experimentally verified both for steady state and for transient conditions using the simulated "clean system". The most important result of this investigation was that the model can predict the transient response of CER measurements very well without the use of adjustable parameters.

The use of the "clean system" allows us to check the validity of carbon balance - CER method of biomass estimation and to test the predictability of the model more readily. The microbial growth model developed from the "clean system" then can be modified by including the hydrolysis kinetic model of lignocellulosics to describe the "real system". The overall model for the "real system" and its experimental verification will be presented in a future report. Such a model will be useful in aiding NASA planners to evaluate the feasibilty

of incorporating the aerobic biological waste treatment system as a component of CELSS.

ACKNOWLEDGEMENT

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PLANT PHYSIOLOGY

Dr. Steven H. Schwartzkopf, Chairman

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CAN PLANTS GROW IN QUASI-VACUUM ?

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The problem of the effect of neutral gas on growth of plants was the motivation of ancient experiments of Schloesing (1897)[1] who observed that nitrogen and argon were not consumed by plants and who concluded that they were probably useless. Since to date we have known that nitrogen can be used by legume plants, but 10 percent of nitrogen is sufficient to supply this process. The question remains to know what happens if, for the same partial pressure of O_2, the nitrogen is suppressed and so, the total pressure falls drastically ? In other words, knowing that only 5 % O_2 (50 mb) is sufficient to maintain plant respiration and that the pressure of CO_2 and water vapour represent around 25 mb in the normal atmosphere, is it possible to conceive of growing plants with only an absolute pressure of 75 mb i.e. a quasi-vacuum ? The predicted answer, following the theory of molecular diffusion of gas is positive but the physiological verification is necessary.

A first positive assay of growth without nitrogen in low pressure was made in the laboratory and mentioned in a space biology meeting (Guérin de Montgareuil et al.)[2], because this theoretical question could also concern space technology. The plant cultivation in space environment is not so far away as we imagined, taking into account the active research of CELSS project (Controlled Ecological life Support System) in which growth of plants and algae is planned for food supply of long distance manned space mission (Moore et al.)[3]. For theoretical and practical reasons it seems useful to analyse experiments in which low pressures were imposed to plantlets by suppression of nitrogen and partly of oxygen.

Materials and methods

Two experiments were conducted in rye-grass and one in barley. They consisted to sow samples of seeds (1 g for rye-grass, 10 grains

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for barley) in vacuum resistant containers in which was prepared different atmospheres.

Containers. Four glass containers were used in each series of experiments, 0.7 liter of volume for the series I, 3 liters for the series II (Fig. 1). In both cases they had leak proof ground joints and stopcock systems to realize the link with a vacuum bench for the initial preparation of atmospheres and to permit the sampling of gas during the experiment.

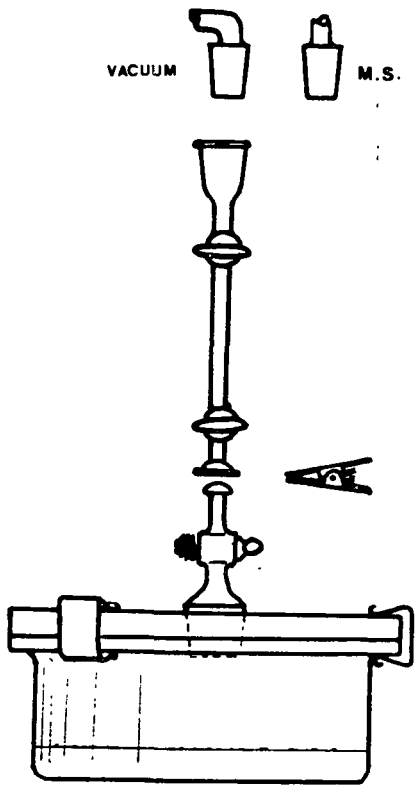

Figure 1. Vacuum resistent glass container for low pressure cultivation of seedling (0.7 liters of volume). On the top, the sampling apparatus which may be fitted on mass spectrometer inlet.

Growing conditions. Each container received 250 g of acid washed quartz sand ; 30 ml of nutrient solution was added and the seeds sown before connection with the vacuum bench. The growth was performed in day light during February (series I) and March (series II) at temperatures between 18 and 22°C. The growth was limited to the juvenile stage because no CO_2 was added and the photosynthesis

only used the CO_2 produced by the respiration, mainly by grain, at the heterotrophic stage.

The preparation of atmospheres with the vacuum bench was realized in three steps. 1) Vacuum actively maintained during 2min around 20 mm of Hg to remove initial atmosphere and to purge dissolved gas in nutrient solution. 2) Closure and observation during 5 min to check any increase of presure above water vapour pressure (19 mm Hg at temperature of 20°C). 3) Adding of different amounts of oxygen. The pressure was measured with a mercury manometer. Expressed in m bar for clarity, they were 48 and 205 mb for the oxygen to correspond to the partial pressures of the concentrations of 4.6 and 20 % respectively in normal atmospheric pressure (1030 mb). In two containers, nitrogen was added until normal atmospheric pressure. Two other containers were kept with only water vapor pressure and the two initial pressures of oxygen. Sampling was made to check the preparation and to follow the composition of atmosphere during the growth.

Sampling an analysis of gas. The sampling of 0.2 ml of gas was made by the pre-vacuum of the volume limited by the stopcocks. For analysis, the sampling tube was fitted on the inlet of the mass spectrometer (MAT.CH4). Standard gas and accurate pressure gauge was used to correct the sensitivity of the apparatus for the different types of gas.

Results and discussion

Visual observations. The main result is that the growth of plants is possible under absolute pressure 14 times lower than the atmospheric pressure (Fig. 2d). In first approximation, plants ignore the absence of nitrogen and only react to the partial pressure of O_2. Hence the growth of plantlets was delayed under low pressures of O_2 in both cases with and without nitrogen. The CO_2 availability being limited by the carbon content of the seed, the final results after 20 days were very similar. The differences in the kinetic of growth can be better observed in Fig. 3 and estimated in the examination of gas analyses.

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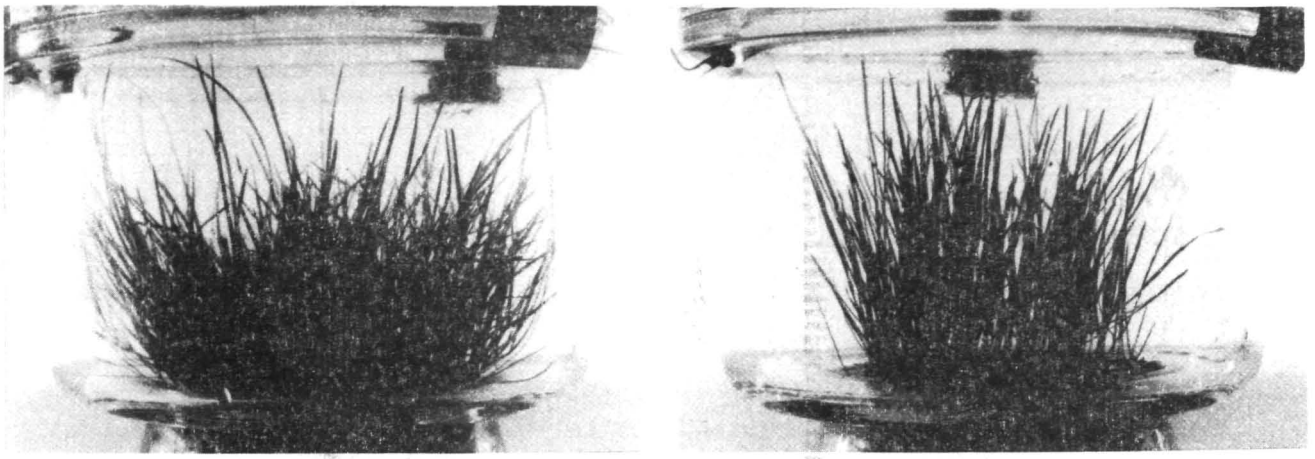
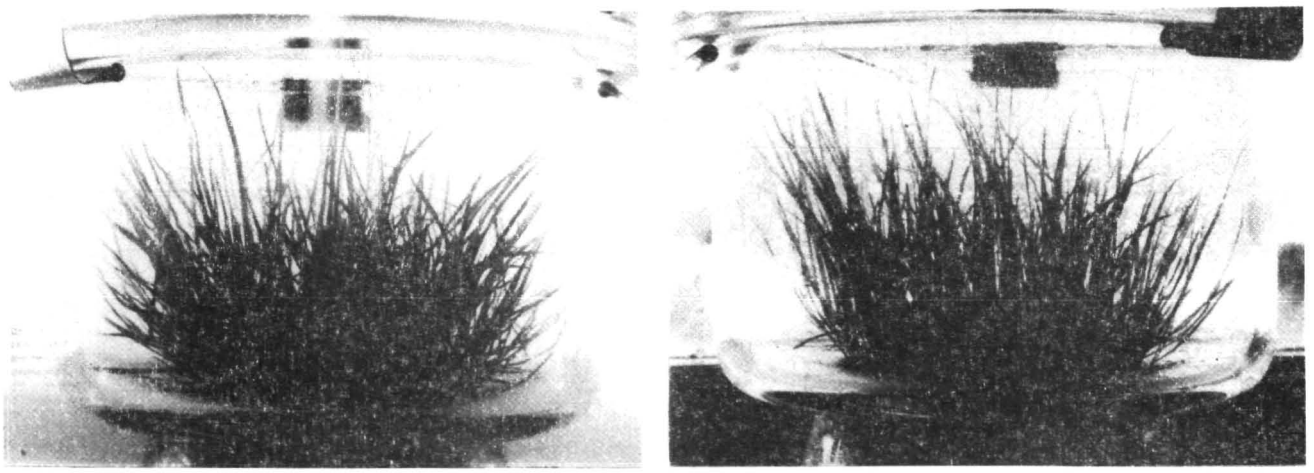
EXPERIMENT AT ONE ATMOSPHERE

20 % O_2 5 % O_2



P. totale	O_2	N_2	H_2O	Partial	O_2	N_2	H_2O	P. totale
985	205	755	25	Pressure	47	916	25	988
230	205	0	25	mbar	48	0	25	73



0,25 Atm. — LOW PRESSURE EXPERIMENTS — 0,07 Atm.

Figure 2. Final result of growing of rye-grass seedling under the different noted pressure conditions. View of the three liter containers 20 days after sowing.

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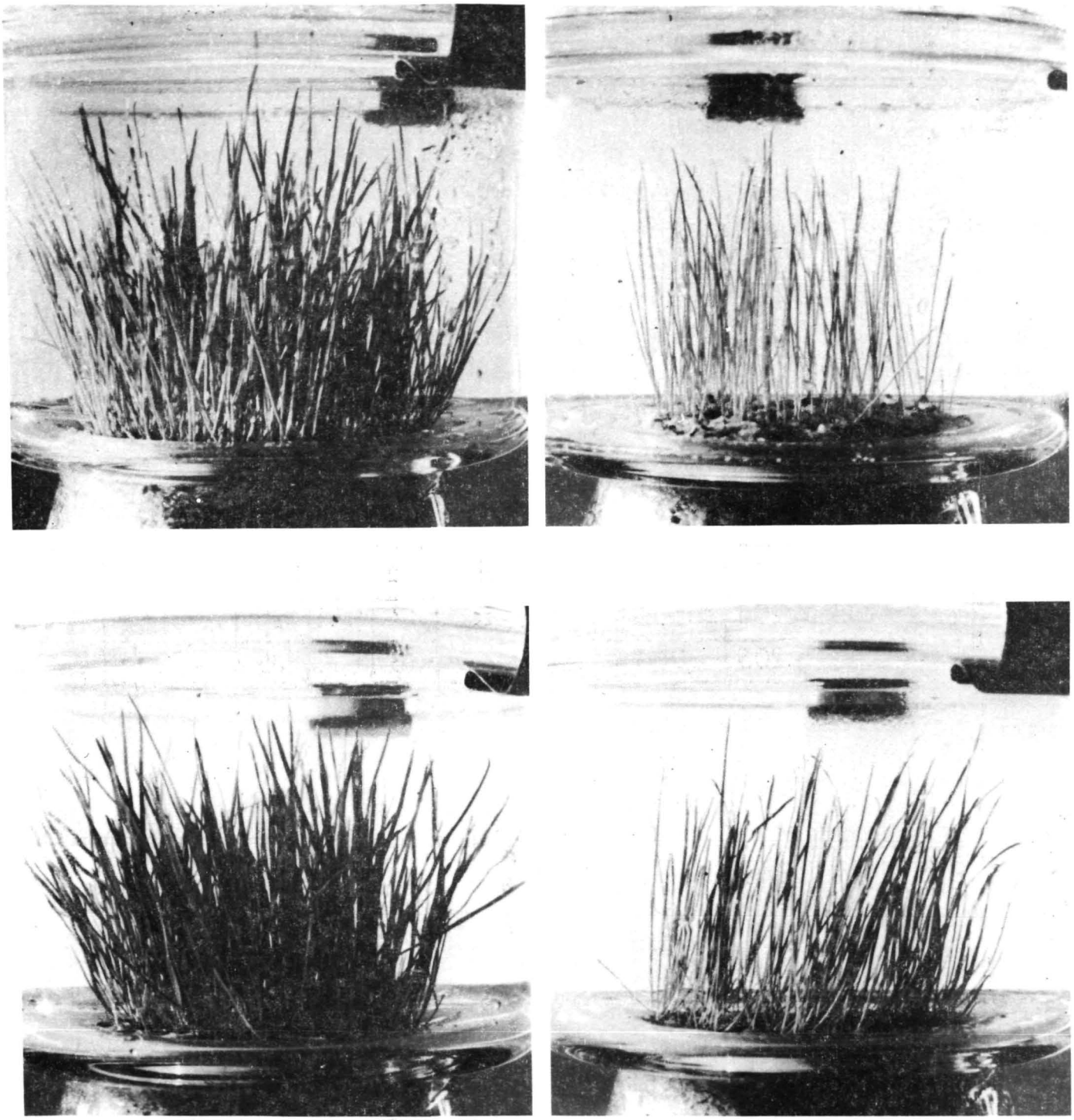

Figure 3. View of the experiment of Fig. 2 - 13 days after sowing. The depressing effect of low O_2 pressure appears(right) and also the depressive effect of nitrogen especially in low oxygen case (right on the top).

In the experiments with barley, only the cases with normal O_2 partial pressure, with and without nitrogen were tested. Seedling was beautiful, no significant differences were observed between both experiments.

Analyses of the atmospheres. Periodic gas analyses have permitted checking the airtightness of the containers. In the case of loss of nitrogen a leak would have been detected by the appearance of nitrogen peak which remained neglectible. Fig. 4 & 5 show the complementary variation of O_2 and CO_2 partial pressures which was amplified in the experiment of Fig. 5 by the effect of the small used volume. Two phases can be observed. In the first phase (0 to 8-11 days) the respiration is the main activity and consequently there is the O_2 decreasing and the CO_2 enrichment. In the second phase, it is the other way round and the photosynthesis re-uses CO_2 faster than it is evolved in a more and more autotrophic way.

The amplitude of the maximal variations was related to the vigour of the plantlets noticed by visual observations in the intermediary phase. The conclusions were as follows :

Slowing down of the growth by low O_2 pressure. Contrarily to the results of Björkman et al.[4] or Quebedeaux & Hardy[5] who observed a growth stimulation on low oxygen conditions, we observed the growth was delayed in the case of low oxygen pressure. The CO_2 and oxygen variation were halved (Fig. 4b, 5) and retarded for one to three days in comparison with normal oxygen pressure. This slowing down can be attributed to a limitation of respiration rate, especially on the grains. Whereas the respiration of the organs such as leaves or roots was generally not modified by a O_2 decrease varying from 20 % to 5 %, the rather strong respiration in particular phases and in not much accessible sites could be limited by O_2 diffusion processes. It was shown by Quebedeaux & Hardy[5] as regarding the phase of the fertilization of the flowers in soybean.

Effect of the presence (of the lack) of nitrogen. If we refer to the visual observations, as well as to the results of gas analyses, the presence of nitrogen significantly slows down the growth of plantlets (Fig. 3).

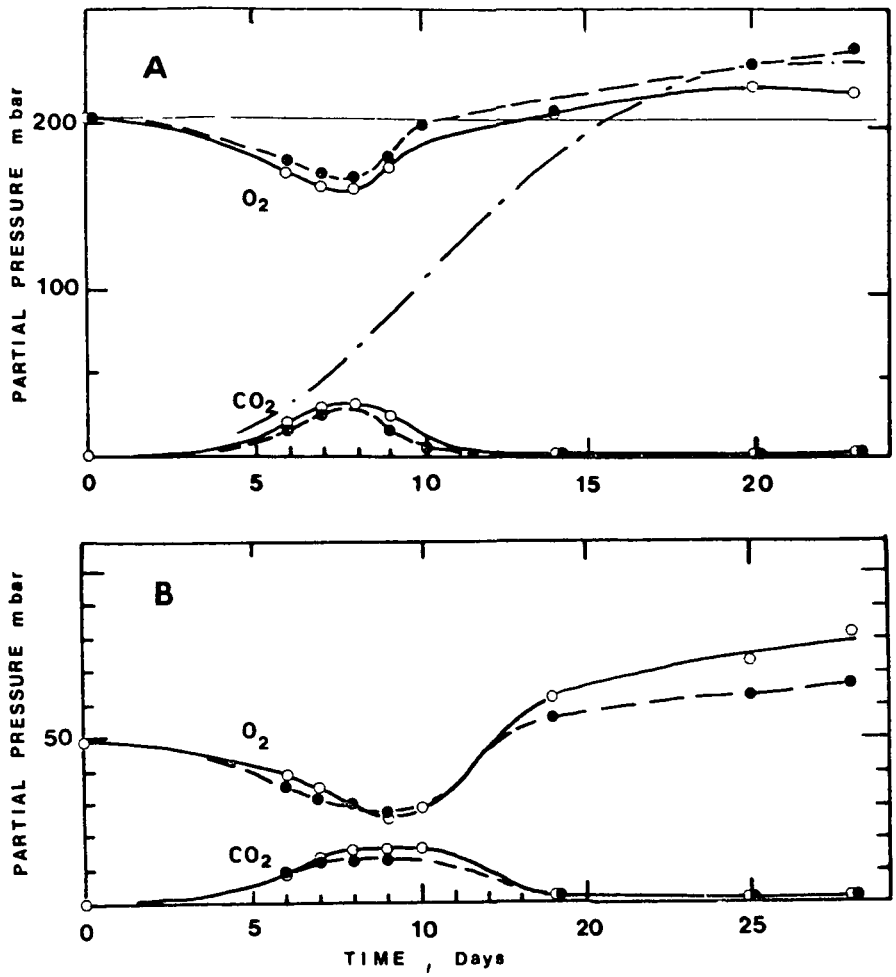

Figure 4. Time evolution of the O_2 and CO_2 pressure in the 3 liter containers. A) With 205 mbar of O_2 initial pressure with (--●--) and without (-O-) nitrogen. B) With 48 mbar of O_2 initial pressure with (-●-) and without (-O-) nitrogen. (— - —) theoretical potential of respiration (see text).

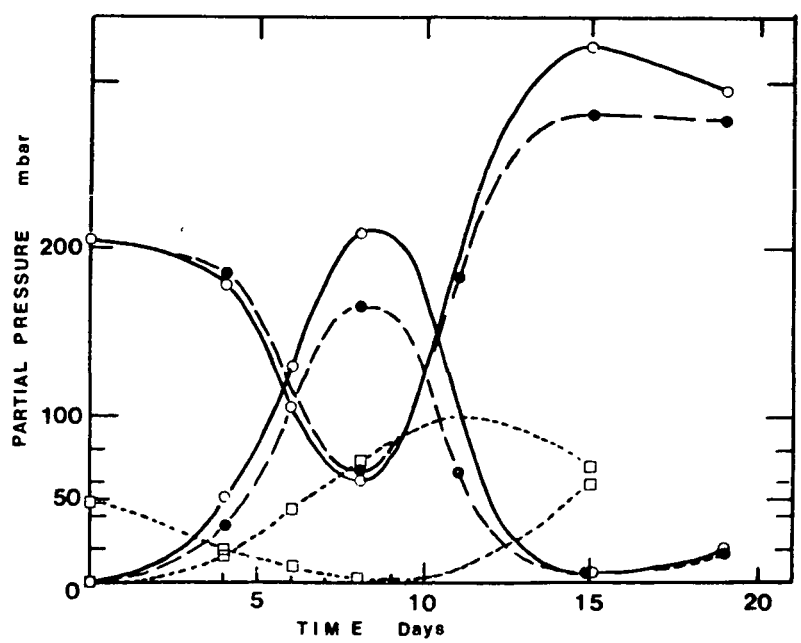

Figure 5. Time evolution of the O_2 and CO_2 pressure in 0.7 liter containers. Same legend as Fig. 4, for 205 mbar of O_2 initial pressure with (-●--) and without (-O-) nitrogen and for 48 mbar of O_2 initial pressure without nitrogen (-·□--).

That is noticeable at a high O_2 pressure, in which the CO_2 maximum is always smaller under nitrogen presence. The slowing down effect of the nitrogen presence is more sensitive in the case of low O_2 pressure and stays visible in the final result (Fig. 2). This effect is surprising. It seems to act upon the number of leaves and of tillers and cannot be explained. However, it renforces the previous idea of this experiment of the "uselessness" of neutral gas.

Disequilibrium of the O_2-CO_2 balance. The present closed experiment is a kind of an environmental microcycle in which is performed the transformation of a system (seed) into another one (plantlet) with conservation of the matter, especially carbon and oxygen. At the end of the experiment, all the components which were at the beginning have to be found. The analysis of the atmosphere shows a significant O_2 enrichment which implies that at the final step the chemical composition of the matter is different, as well as its redox status. The plantlets were in a more reduced state than seeds. This status implicates an energetic gain brought by light for, on the contrary, the final state of a biological process taking place without energetic supplies would be necessarily more oxidized, i.e. with less free oxygen.

Given in terms of concentration, the excess of oxygen seems important, especially in Fig. 5 ; given in volume it is the same : 70 ml for this experiment and between 60 to 90 ml for the experiment of Fig. 4. These values have to be compared not to the oxygen content of the atmosphere but to the turn over of the cycle of transformation mentioned above. For that, the hypothesis is given that the matter of the seeds has been totally transformed and that its structure is - in majority - the carbohydrate one. In that case 1 g of seed correspond to 0.033 mole of CH_2O. Thus we can notice that the excess of oxygen (3.1 m moles) represents about 10 % of the turnover of the total organic matter. Let us notice too that if the CO_2 variation is due to a respiratory activity, it is far from representing the totality of the respiratory potential of the seeds. If all the dry matter of the seeds was consumed by respiration, the hypothetical curve plotted on Fig. 4a could be obtained. It is not excluded that a large part of this matter was thus degraded, but in that case CO_2 was immediatly

trapped by photosynthesis and the observed curve results from the equilibrium between the two processes.

Possible consequences for culture in space environment.

Safety this experiment demonstrate that plants are, in first approximation, insensitive to de-pressurization. Only the loss of water, in case of active vacuum, would be crucial.

Space technology. As far as this simple experiment can be generalised, two types of consequences can be suggested. If the cultivation of higher plants must effectively supply a noticeable part of food of future space stations a considerable volume will be devoted to it. Take account of the environment of the space vacuum, the possibility of culture under low pressure proportionally reduces the losses of gas due to unavoidable leaks. But above all, this proceedure reduces the quantity of material required to face vacuum constraints. For the same weight (cost of launching) the gain of volume can be (at least) proportional to the reduction factor of the pressure. If a factor of ten can be expected, this process will be certainly discussed in the future in spite of the great disavantage of space clothes for space gardners.

Advantage in O_2 recycling for ecological system. The elimination of all or part of nitrogen in a cultivation system (as well for higher plants and algae) drasticaly simplifies the management of its atmospheric phases and the control of the oxygen loops between cultures and manned rooms. Cultures are supplied by CO_2 trapped in maned area. O_2 production increases the pressure in culture systems and the its extraction is made by the pump which maintain the depressurization. Dilution process are suppressed. Pure or very enriched oxygen is produced without nitrogen separation. It can be stored for the reserves required by the control of O_2 cycle or to supply the missions out of the space station.

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WHEAT RESPONSE TO CO_2 ENRICHMENT : CO_2 EXCHANGES TRANSPIRATION AND MINERAL UPTAKES

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Introduction

In long term application of CO_2 enrichment (i.e., from two to ten times the normal concentration) an increase in the dry matter production has almost always been observed. For C_3 plants the enhancement of dry weight ranges from almost a 5 fold increase for soybean [1] to barely a 1.2 fold increase for wheat [2], [3], rice [3] and tocacco [4] with many intermediate values (table I). Kramer's review [14] posed tentative generalization about growth responses of plants to enhanced concentration of CO_2 : I) large differences exist among various species ; II) responses are greater with indeterminate plants than determinate ones ; III) the largest response occurs in seedlings and decreases or ceases as plants grow older. The two first propositions are not strongly proved and studied and the effects of difference or not well defined conditions could explain much of observed variations. So, the Kimball's review [15] situates the average of stimulation coefficient of dry matter production, for a doubling of CO_2 level, in the moderate range of 30 - 40 %.

Ever since the Athens conference [16], more attention has been paid to studying the crop and natural vegetation response [17] and to developing models predicting dry matter and yield enhancement. Progress in the crop studies was obtained by the experiments of Rogers et al [18] (with open-top chambers) and of Jones et al [19] (with day light growth chambers). But the discrepancy between their results concerning the dry matter enhancement factor (for example when doubling CO_2 one obtains 1.63 and 1.36 respectively for the same soybean plant) still remains to be explained.

As for proposition III) by Kramer, it was based on the decrease of the response to CO_2 enrichment with the length of treatment, as reported by Ford and Thorne [6] for barley, Neals and Nicholls [22] for wheat and Patterson and Flint [12] for soybean. It poses a problem for quantitative predictive models and asks questions for plant physiology : increase of

Matériel végétal	Réf.	Teneur en CO_2 ($\mu l\ l^{-1}$)	Durée de traitement (j)	Rapport fort CO_2/CO_2 normal	Nombre de plantes par échantillon	Eclairement ($\mu Em^{-2}\ s^{-1}$)	Temperature (°C)	Photo-période
Blé	5 *	490	140	1,32	20	LN (été)	21/16	8/16
	2 *	950	28	1,47	6 à 12	ca 1120 (a)	15°6 (a)	16/8
	8	600	17	1,30	10	360	22°5/20	20/4
			24	1,13				
	10 *	675-1000	47	1,90-2,16	10	600	26/20	12/12
Betterave à sucre	13	1000	10	2,80	10	320	28/22	16/8
	4	1000-3300	21	1,26-1,25	7	ca 330	20/15	16/8
			42	1,23-1,23	7			
Chou frisé	4	1000-3300	21	1,44-1,50	7	ca 330	20/15	16/8
			42	1,48-1,39	7			
Coton	12	640	40	2,00	5	LN (été)	32/20	
	7 *	630	110	2,10	9	LN (Mai-Août)		
Maïs (C4)	6	1000-3500	15	1,06-1,14				
	12	640	30	1,20	8	LN (été)	32/20	
	9	600-1000	12	1,17-1,21	8	650	28/22	14/10
			24	n.s. - n.s.				
			45	n.s. -0,86				
Orge	6	1000-3500	15	1,36-1,23	42	ca 330	20/15	16/8
	4	1000-3300	21	1,44-1,61	42			
			42	1,28-1,24				
Riz	6	1000-3500	15	1,24-1,23				
Soja	7 *	630	110	4,80	9	LN (Mai-Août)		
	9	600-1000	12	1,53-1,80	8	650	28/22	14/10
			24	1,30-1,77				
			45	1,23-1,72				
	1	1000	9 -35	1,84	8	600	26/20	12/12
	3 *	1350	38	var. Chippewa : 1,51 var. Hark : 1,67	2	ca 280+LN(Fev-Av)	30/32	14/10
Tabac	11	1000	6 - 24	2,42	15	ca 780	26/22	9/15
			6 - 45	1,35	1			
			31 - 45	1,20				
Tournesol	7 *	630	110	1,60	9	LN (Mai-Août)		

Références : 1 : Clough et Peet 1981 (5) 2 : Combe 1981 b (9) 3 : Cooper et Brun 1967 (11)
col. 2 4 : Ford et Thorne 1967 (6) 5 : Gifford 1977 (10) 6 : Imai et Murata 1976 (3)
7 : Mauney et al. 1978 (1) 8 : Neales et Nicholls 1978 (2) 9 : Patterson et Flint 1980 (12)
10 : Sionit et al. 1981 d (7) 11 : Thomas et al. 1975 (4) 12 : Wong 1979 (13)
13 : Wyse 1980 b (8)

Table 1 : Effect of CO_2 enrichment on the dry matter production. The col. 4 gives the enhancement factor.

n.s. : non-significant effect ; (a) average on 4 weeks ; * : until maturity ;

LN : natural light.

carboxylation is obtained at the expense of the oxidative photosynthetic carbon cycle. Measured by $^{18}O_2$ uptake in C_3 plants, this cycle consumes as much reducing equivalents as reductive carbon cycle [20]. It may have some physiological use for example by the well known coupling with nitrogen metabolism. When it is repressed, that can explain, together with many other already evocated processes [16], the decrease with time of the growth efficiency.

Long term effect of CO_2 enhancement also concerns research in the photorespiration genetics [21], because the permanent change of CO_2 exactly simulated a pure genetic change in the specificity factor of CO_2 of the Ribulose bisphosphate carboxylase/oxygenase.

Hence, to help understanding the physiological control of dry matter production posed in new and pertinent terms by CO_2 effect studies, several experiments were performed on wheat, by integrating gas exchanges at the whole plant and canopy level [22], [23]. Particular attention was paid to I) growth response from seedling to dense canopy situation [24] ; II) change in photosynthetic and photorespiratory characteristics at single plant or canopy level [25] ; III) water use efficiency in normal and limited watering ; IV) Relation between daily CO_2 uptake and mineral uptakes. We'll report here typical results of this study, with emphasis on data which are not yet published.

MATERIAL AND METHODS

Plant material and growth conditions

Wheat seeds (*Triticum aestivum* L., var. Capitole) were germinated between wet filter papers in the ambient conditions of our laboratory (temperature was 20°C $\pm$ 1). No chilling treatment was applied for floral induction ; so, tillering went on throughout the experiment. Three day-old seedlings were planted out in pots of 1.45 liter : 5 and 1 plant per pot in the high and low density experiment respectively. The plants were grown either on garden soil or sand.

The day after planting, 20 pots were distributed on a surface of 0.5 m^2 in each of two C23A* twin growth chambers [26], [27], [28]. The CO_2 concentration was regulated at 330 $\pm$ 5 $\mu l\ l^{-1}$ in the control chamber and at 660 $\pm$ 5 $\mu l\ l^{-1}$ for the high CO_2 treatment. The other climatic

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*C23A= Chambres de Culture Automatique en Atmospheres Artificielles described by Andre et al in the session on International CELSS

conditions were the same in both chambers. The photon fluence rate, provided by 3 Osram HQI-T 400W/DV lamps, was 600 ± 90 µmoles $m^{-2}s^{-1}$ at a height of 30 cm from the bottom of the chamber. Air flow (rate of 0.4 - 0.6 ms^{-1}) was vertical from a grid on the floor of the growth chamber. The temperature and the relative air humidity were respectively, 24°C, and 50% during 14 hours of light and 18°C and 85% during 10 hours of darkness. The four walls of the chambers were covered with polished Aluminium sheets in order to reduce the irradiance border effects. The homogeneity of climatic conditions inside each chamber had been tested previously [29, 30] : on a horizontal plane the deviation was less than 4% for temperature and less than 15% for light. Four different treatments were carried out by crossing two CO_2 concentrations (control and high) with two plant densities, in order to obtain different light availabilities (200 and 40 plants m^{-2} for high and low density respectively). The plants were grown in controlled conditions until almost 40 days after germination. The experiments described in this paper, constitute a part of a series of six, twin experiments on CO_2 enrichment achieved in similar or identical conditions, at least for 25 days. At that stage the influence of a limiting water supply has been studied in part of them. These experiments included the inversion of the CO_2 concentrations in the two chambers and showed the reliability of the system. Results were reproductible within 15% for CO_2 gas exchanges for successive crops. The accuracy of relative measurements, between twin chambers, was better than 5%.

CO_2 gas exchange measurements

The CO_2 concentration was measured with an infra-red gas analyser (Hartmann and Braun-URAS 2T). The CO_2 uptake by photosynthesis and the CO_2 release by respiration during the night were compensated respectively by calibrated CO_2 injections and CO_2 trapping with soda lime. The system has been previously described [31]. A computer (Télémécanique 1600) anticipated the compensation of the gas exchanges between two consecutive gas measurements (about every 7 minutes), in order to reduce the deviation from the set point. The same computer recorded the physical parameters (light, air and dew point temperatures) and the amount of CO_2 injections or the time of CO_2 trapping so that photosynthesis and respiration could be easily deduced. All the calculations were achevied with a Solar 16-40

mini-computer. During daytime the O_2 concentration was kept normal (around 20.6%) in spite of the photosynthetic O_2 release, by using a gas mixture of 80% N_2 and 20% CO_2 for the CO_2 injections.

Photosynthesis (which included the CO_2 uptake of shoots minus the CO_2 evolution from the roots) and respiration were mean values of the CO_2 gas exchanges of the canopy. A correction for soil respiration was applied when garden soil was used. This value was estimated from a preliminary experiment on pots without plants in the same chambers (9.0 mg CO_2 H^{-1} during the light period and 6.1 mg CO_2 h^{-1} pot^{-1} during the night). Over the whole period of growth this correction accounted only for 6% and 7.5% of the final dry weight at 660 and 330 µl l^{-1} respectively.

Area of leaves and growth measurements.

During the high density experiment we harvested a sample of ca.15 plants randomly chosen at 23, 30 and 37 days after germination. In the low density experiment only two harvests took place at 23 and 38 days, of 5 plants each. After sampling the remaining pots were reorganized in order to avoid the formation of gaps in the canopy.

At each harvest, the length of the lamina was measured. A correlation curve between the length of the lamina and their surface (as determinated with manual planimeter) had been established previously in order to obtain leaf lamina surfaces. These were taken as leaf surfaces. Leaf area index* was calculated by the product of leaf area per plant by the number of present plants. A leaf was considered to be expanded when the ligule was visible. The plants were dried in an oven at 65°C during 60 hours before their different parts were weighted.

The continuous growth curves were obtained (without any fitting) by cumulating the net daily photosynthesis (PN) to the initial seed weight (52 mg). The factor relating the integration of PN expressed in mg of CO_2 per plant to the dry weight in grams per plant, was determinated by considering a dry matter carbon content of 44%.

Transpiration and mineral consumption

Methods previously described [31], [32], were performed with sample of 20 pots and in sand culture. The daily watering with nutrient solution (Hoagland and Arnon n°2) provided about twice as much water as was needed for transpiration. A device connecting each pot to an individual and

* leaf area index (LAI)

calibrated reservoir, provided a good uniformity of the solution supply and allowed an easy control of the working of the watering. Tests of the system, with 40 pots, gave a maximum deviation of 4% from the wanted volumes. Weighting of the water, condensed in the cooling system kept at the accurate wanted dew point, gives the daily transpiration.

The collected overflow of watering was also weighted, pH controlled and kept in fridge for analysis. Nutrient uptake of NH_4 - NO_3 - P - K, was obtained from multichanel Autoanalyser Technicon data. Computer monitoring and data processing made comparison with fresh solution, calibrations with standard solutions and calculations of uptakes take into account imput/output of volume and concentrations [27], [32].

RESULTS AND DISCUSSION

Growth rate analysis

Continuous monitoring of CO_2 exchange provided continuous growth curves of carbon accumulation (Fig.1) and accurate relative growth rate* data reported elsewhere [24]. The main results for growth models was that : I) *a little effect of CO_2 on RGR was noticeable in first 14 days but then* disappeared ; II) The expected exponential amplification of CO_2 effect [33], [16] was not observed. Quasi exponential kinetics was observed but related by constant ratio and so, with same R.G.R. at the same time ; III) Reciprocally a consequence is that the R.G.R. comparison (as for example Rufty's study [35]) is not the pertinent measure of a growth stimulation ; IV) Higher apparent stimulation effect in early stage previously reported [23] was, after analysis, attributable to the artefactual seed matter respiration : when, from negative figure, the carbon balance begins positive, a little advantage of one treatment can give an infinite ratio of stimulation. This effect was corrected [24] and the stimulation factor naturally rises from 1 (initial status of seeds) to finite values (Figure 1) (only in that phase R.G.R. was little stimulated).

Effect on carbon accumulation.

In order to follow the time-evolution of the effect of CO_2 enrichment on dry matter production, we plotted in figure 1 the ratio of high CO_2 to control growth curves as deduced from CO_2 exchange measurements.

Growth was followed in two distinct ways : by harvesting plant

*relative growth rate (RGR)

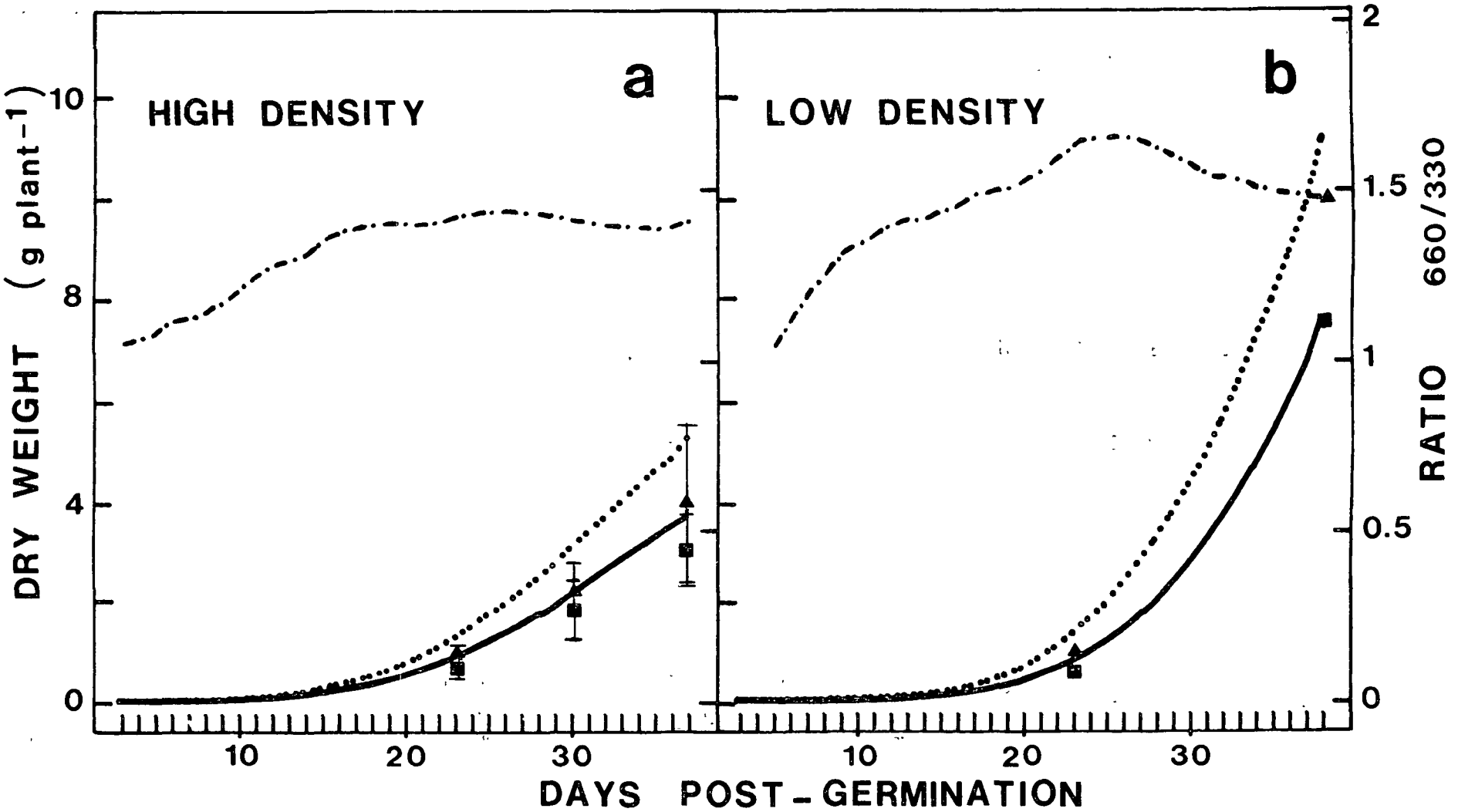

Figure 1 : Continuous growth curves obtained from the integration of the net daily CO_2 uptake of whole canopies :
(———) 330 µl l^{-1} CO_2 and (.....) 660 µl l^{-1} CO_2. Ratio of high CO_2 to control growth curves (-.-.-) ; dry weights from harvests (samples of 15 and 5 plants respectively in the high and the low density experiment) : (■) 330 µl l^{-1} CO_2 and (▲) 660 µl l^{-1} CO_2. bars : standard deviation.
a : 200 plants m^{-2}. b : 40 plants m^{-2}.

material at time intervals, and continuously by the daily net carbon uptake. As shown in figure 1, the results did not exactly agree. Part of the global deviation might be ascribed to the uncertainly about the real carbon content of the dry matter.

The ratio between the continuous growth curves of both CO_2 treatments (Figure 1) was higher and more constant than those obtained from dry weight measurements. The most probable explanation for the discrepancy, was the large variability of the dry weight measurements, even with the largest samples (10 to 15 plants) and in spite of the attention paid to a good uniformity of the growth conditions inside each chamber. As a consequence, even the difference between samples from the two CO_2 levels was not always statistically significant. So, we will prefer the data obtained from CO_2 exchanges to discuss the factor of stimulation.

At both densities this ratio rose during the first 15 days up to a level of ca. 1.45 for the high density and ca. 1.50 for the low density. For the high density it then remained almost constant until the end of the experiment. However, for the low density, the ratio rose significantly (up to 1.65) until 23 days, stabilized a few days. That was due to the compound of two effects : relative increase of surface (by 20%) and promoting effect of CO_2 on photosynthesis. When leaf area increased (mainly due to tillering), the advantage of surface decreased. The ratio dropped again from the 26th day to ca. 1.50. In spite of a subsequent rise of LAI to 9.4 at 330 $\mu l\ l^{-1}$ CO_2 and to 10.3 at 660 $\mu l\ l^{-1}$ CO_2, the ratio was maintained at this level until almost 70 days.

Thus final effect of the high CO_2 treatment on dry weight per plant (1.45 for high density and 1.50 for low density) was not much influenced by the plant density.

The promoting effect of CO_2 enrichment on dry weight of wheat seedlings showed no decreasing tendency with time (Fig.1). In contrast, Neales and Nicholls [2] found a progressive decrease of the stimulation of biomass at high CO_2 for young wheat plants too. The discrepancy might be explained by the difference in our respective photoperiods. Neales and Nicholls [2] applied a very short night (4 Hours) which might have resulted in a subsequent increase of the concentration of assimilates in the leaves, especially for the high CO_2 treated plants. A reduced photosynthetic capacity has often been observed in such conditions (Neales and Incoll [34]).

For both densities, the final enhancement of dry weight by CO_2 enrichment (1.45 at high density and 1.50 at low density) was in the same order of magnitude as the results reported for wheat by Gifford (1977), Combe [9]. However, the low density experiment showed a transient stimulation of 1.65 at 23 days for a LAI below 1, i.e. for strictly isolated plants. In the same way Sionit et al [7] found a two fold increase of dry weight with 675 $\mu l \cdot l^{-1}$ CO_2. Even if no plant density was mentioned in that paper, we can assume that plants did not drastically interact. In the same way, the difference of behaviour between an isolated plant and a canopy might explain some discrepancies in the literature about CO_2 enrichment evocated in introduction. For instance, Rogers et al [19], reported at either 620 or 815 $\mu l\ l^{-1}$ of CO_2, respectively a 1.63 and a 1.77 fold increase of soja biomass in field experiments with a density estimated around 7 plants m^{-2}. In contrast, Jones et al [19] obtained a stimulation of 1.36 and 1.53 respectively at 600 and 800 $\mu l\ l^{-1}$ of CO_2 in out-door chambers but with 50 plants m^{-2}. It seems probable that the former observed the effect on relatively isolated plants when the later better simulated a crop canopy.

The difference between the response of an isolated plant and a canopy to CO_2 enrichment can be explained by the growth components involved in the buildup of the gain of carbon. In the case of an isolated plant (or LAI below 1), the stimulation of dry matter production results from promoted Net Assimilation Rate (NAR) and leaf area. Furthermore, the impact of the gain of leaf area on dry matter production depends on the duration of the phase in which LAI stays below 1. Therefore we found no such transient of high level of stimulation in the high density experiment.

In contrast, for a closed canopy (LAI beyound 2-3) the response of crop assimilation (per unit of ground area) to the increase of LAI is almost saturated [24]. The stimulation factor no longer depend on leaf area increasing. It depends only on the response of the canopy (considered as a big leaf) to the CO_2 enhancement. Only the second order processes (i.e. change of stomatal conductance, regulation of carboxylation rate, photorespiratory and respiratory changes) can intervene.

Comparison of long-term with short-term responses of photosynthesis

Daily Photosynthesis and respiration of cultures with and without

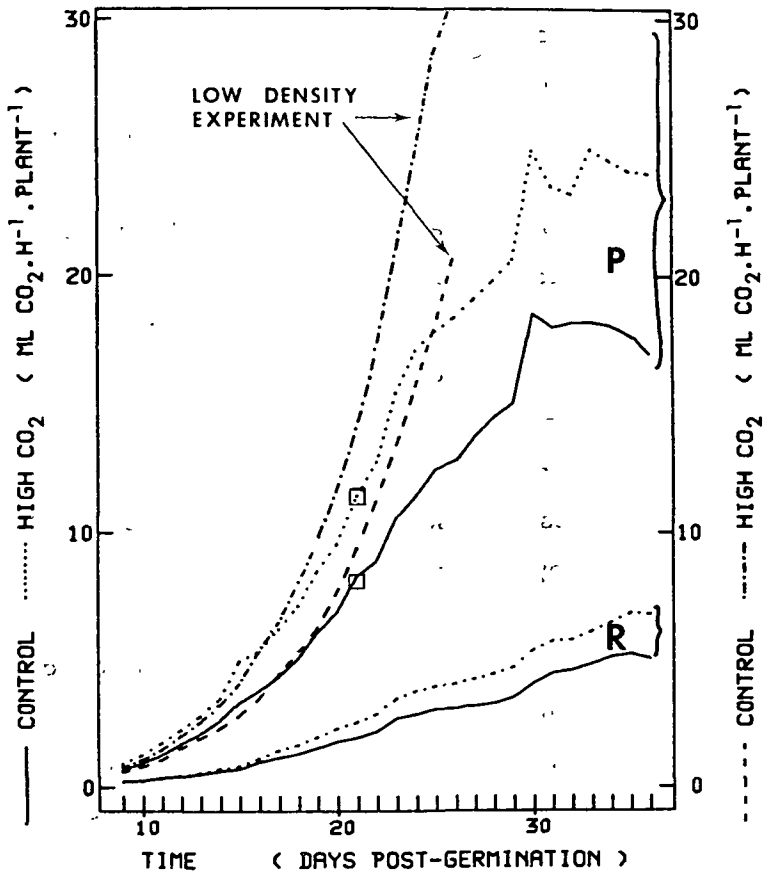

Figure 2 : Long term effect of CO_2 enrichment on daily photosynthesis (P) and respiration (R) expressed in mean rate of 14 hours of light period, and of 10 hours darkness respectively. High density experiment (———) 330 and (-----) 660 µl l^{-1} of CO_2. P of low density experiment (-----) 330 and (—.—.) 660µl l^{-1} of CO_2.

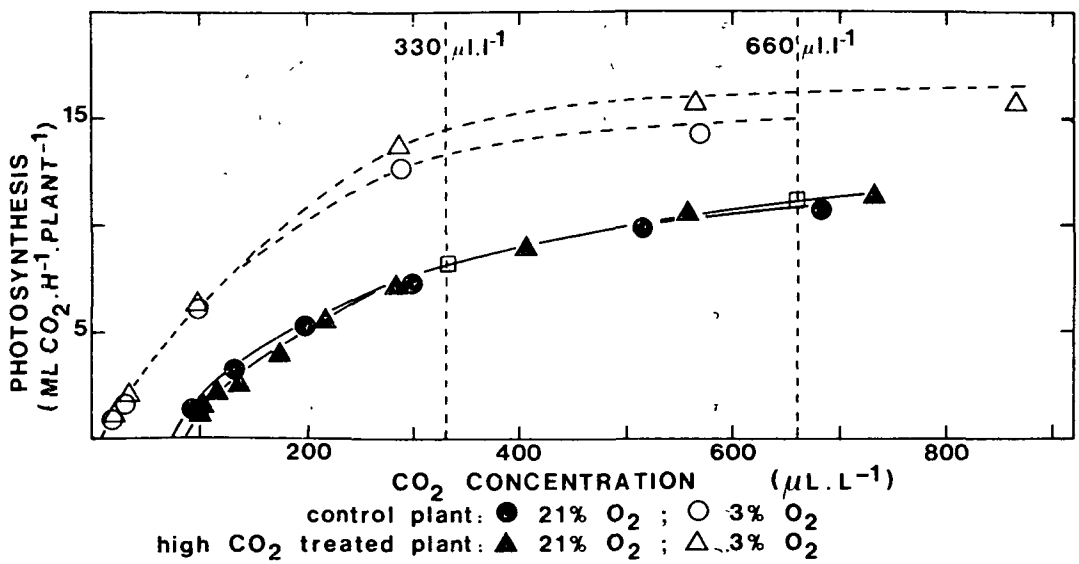

Figure 3 : Typical short term response of the photosynthesis of wheat canopies. Measurements were made at midday of the 21th day in the high density experiment of Fig. 2. Rates of photosynthesis were very similar for both canopies (LAI around 2) and particular values (□) well correspond to average values of Fig. 2.

CO_2 enrichment are shown in Fig.2. They were used to calculate the long-term effect of CO_2 on dry matter production discussed above. Second order effect can be estimated if one permutes the atmosphere or if one plots (Figure 3) the short term photosynthesis response of each canopy to the CO_2 concentrations. Such curves were systematically obtained in both crop densities when LAI was equal or above 2. At first they indicate that the photosynthetic characteristics (for normal O_2 pressure) of the two crops were similar (same big leaf) and therefore the second order of regulation processes were neglectible or compensated each other. Crossing the atmosphere gave also the same photorespiratory rate [25]. Only a carreful analysis of photosynthesis (corrected to correspond to exactly the same LAI) suggests a second order effect which reduces the photosynthetic capacity by about 5 to a maximum of 10 per cent [22, 24].

The second observation (also consequence of previous consideration) was that, if we double the CO_2 concentration on the reference experiment growing at 330 $\mu l\ l^{-1}$, we obtain the same photosynthesis as the high CO_2 adaptated sample, and furthermore the values fit the daily average values (Fig.2) of the corresponding day. In other words, short term experiment can be used to predict long term response to CO_2 of the crop canopy production. If such behaviour is verified for other plants the consequence should be important to predict first order response of dry matter accumulation of vegetation in closed canopy. Experiment of Jones et al [19] in Soybean, by crossing the CO_2 level suggest similar conclusion in accordance with moderate enhancement factor and absence of a negative feed-back regulation.

The advantage given by CO_2 enrichment to obtain productive canopy (LAI > 1) faster than in normal conditions is neglectible in our case. The treated culture was only one or two days in advance in the kinetic of area increase.

Effect of CO_2 on transpiration

Transpiration rate at a given time were very similar for experiment without and with CO_2 enrichment (Figure 4). Taking into account the difference of leaf area (less than 20%) but also the non-linear relationship between transpiration and LAI (which forbid simple normalization by surface) a little effect of reduction of stomatal conductance (5%) was estimated for the same LAI[22].

The water use efficiency was increased from 10 to 14 mg CO_2 g-1 H_2O (Fig. 5A) .

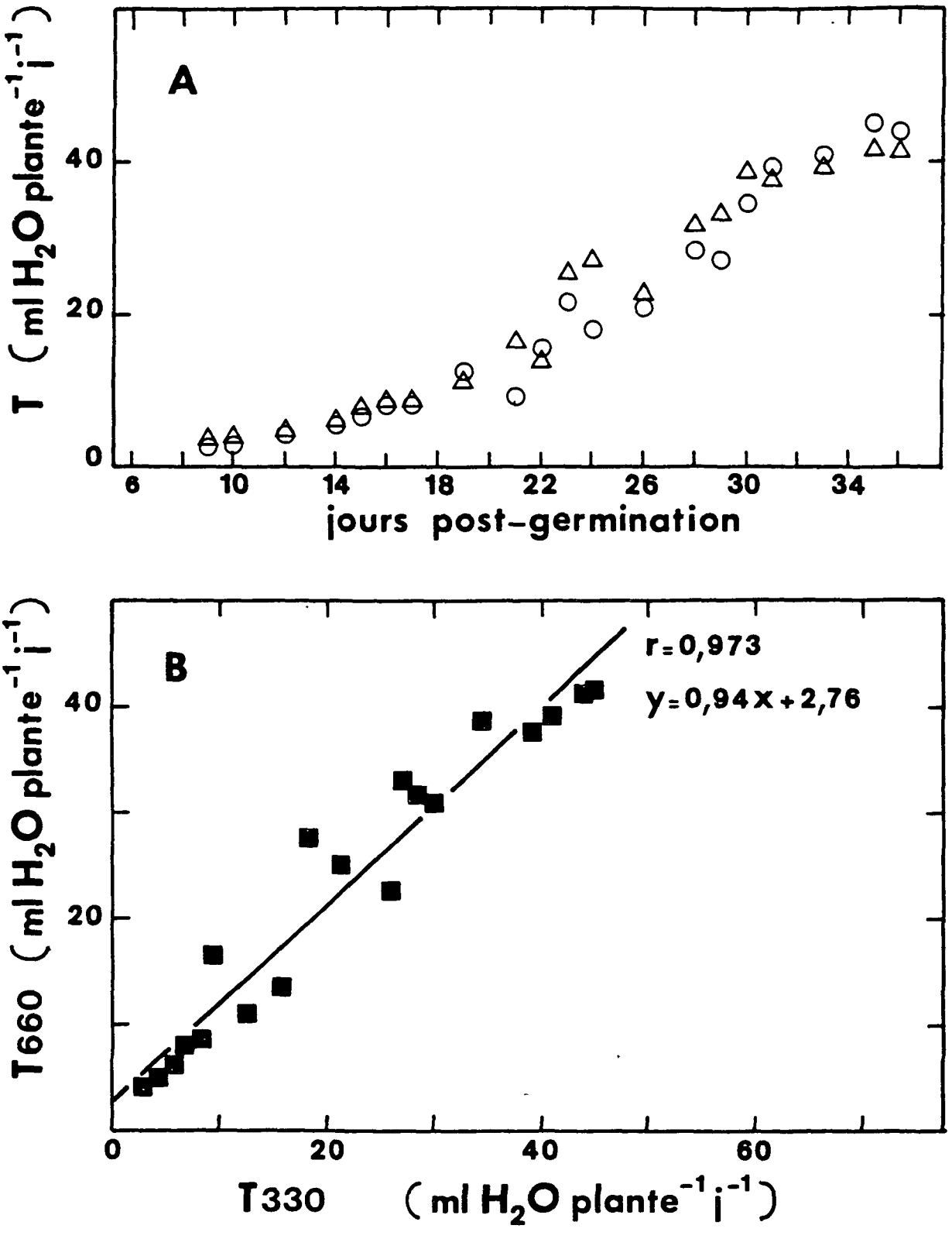

Figure 4 : *Effect of CO_2 enrichment on transpiration.*

A) Time evolution of the daily transpiration of the high density culture with (○) 330 and (△) 660 µl l^{-1} of CO_2. Same conditions as Fig. 1.

B) Correlation between the daily transpiration of the two cultures at the same time.

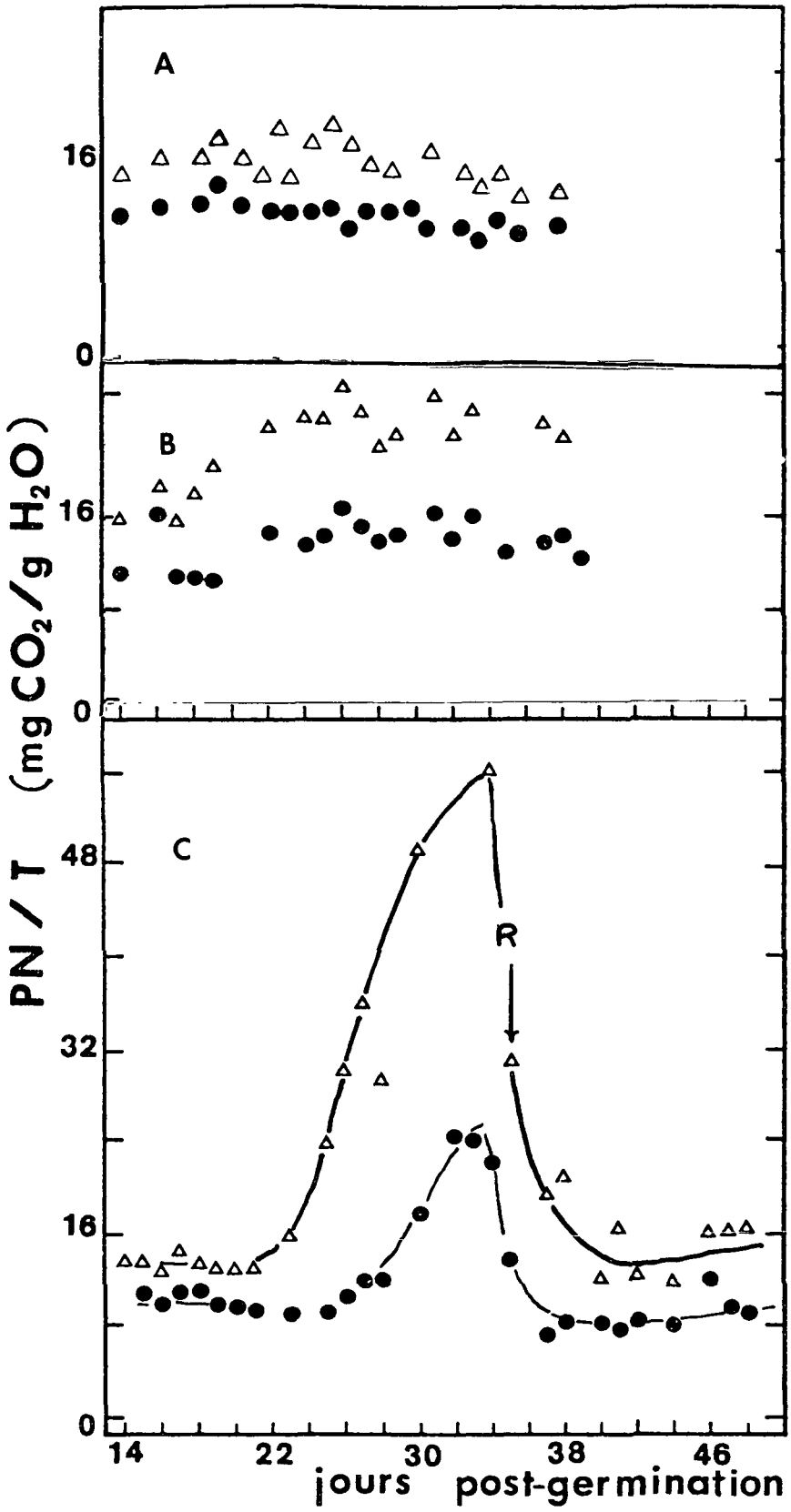

Figure 5 : Water use efficiency of two cultures at high density at (●) 330 and (Δ) 660 µl l^{-1}.

A) Without water limitation (average of two experiments)

B) With the limitation of 20 ml day^{-1} $plant^{-1}$, and (C) of 10 ml day^{-1} $plant^{-1}$ of water (Experiment of Fig. 6)

(R) Rewatering.

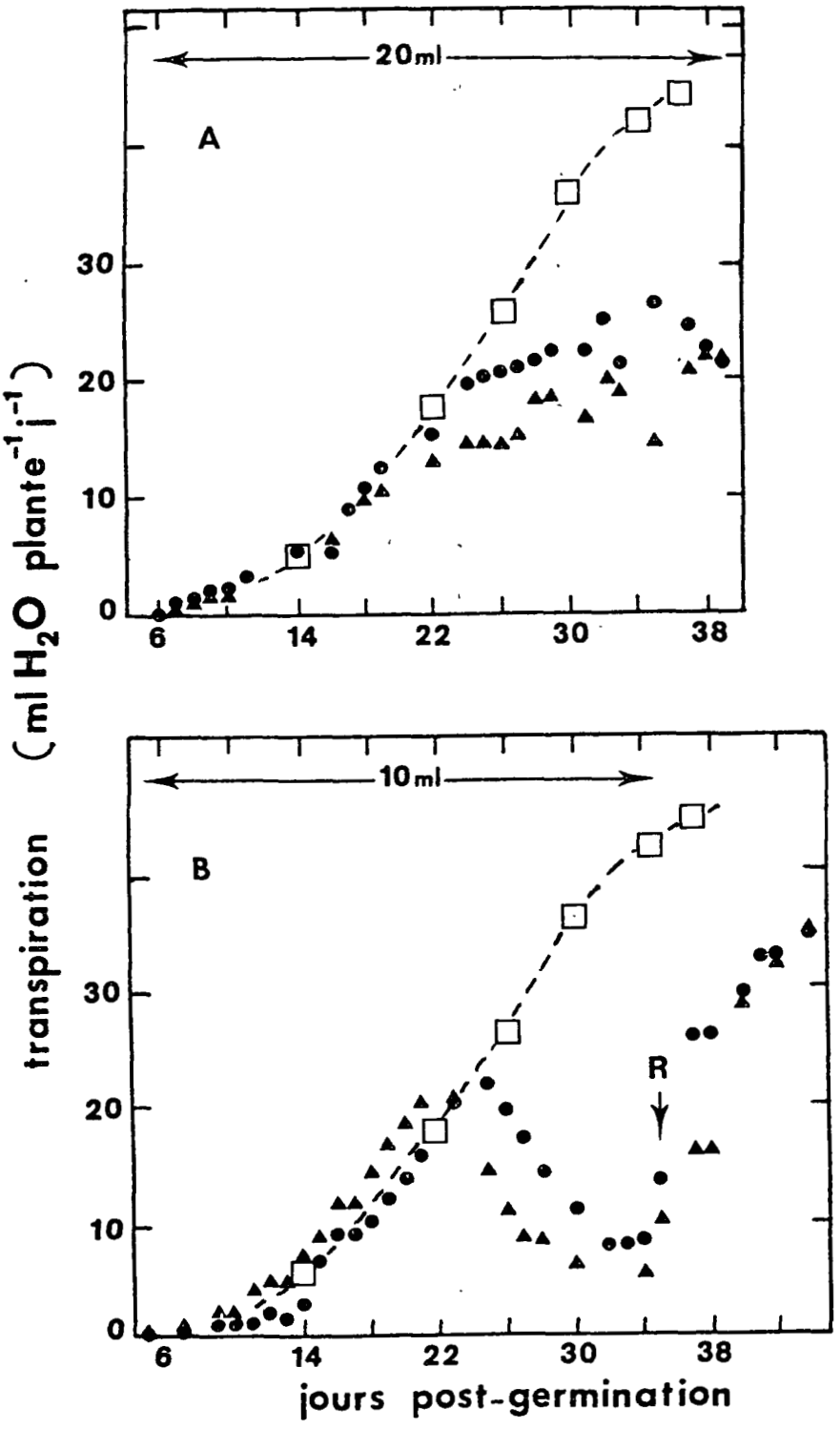

Figure 6 : Effect of moderate water stress on the transpiration rate of two cultures at high densities with (●) 330 and (Δ) 660 µl l^{-1} of CO_2 ; (□) standard well watered experiment. The watering was limited to (A) 20 ml day^{-1} $plant^{-1}$ and (B) 10 ml day^{-1} $plant^{-1}$ until 37 days, then rewatered (R).

Water limitation.

When watering was limited to a given value (20 or 10 ml day^{-1} $plant^{-1}$) - simulating low but constant water availability - the dry weight production was reduced by 43% and the leaf area by 57% at day 35 in the later case. Figure 6 shows that the output of transpiration raises above the input of watering during the depletion of the water storage in the pot (estimated by weighting of 350 ml, sand and bottom reserve). The transpiration finally falls to reach the level of watering. So, at day 35, for the normal CO_2 experiment the transpiration was five times lower than the well watered experiments. Daily photosynthesis was only halved and water use efficiency was more than two times higher. With CO_2 enrichment the photosynthesis is less reduced and the water use efficiency raises up to 56 mg $CO_2.g^{-1}$ H_2O i.e. around five times the well watered standard (Fig. 5c).

In both cases during the water stress the xylem's hydric potential changes from - 3 to - 6 bars in normal CO_2 and from - 5 to -8 in high CO_2 level. The later one having a constant difference of potential in agreement with results of Sionit et al[36].

These observations confirm, and quantitatively illustrate, the general agreement on this subject [16]. They respond to the need to link observations made on leaves - the most often reported - and the crop canopy behaviour.

Effect of CO_2 on Nutrient uptakes.

The daily measurement of mineral uptakes in large sample of plants was succesfully performed. Figure 7 shows that at a given time the consumption of nutrients was similar in normal and high CO_2 culture. So, as the photosynthesis was higher, the mineral uptake was relatively lowered in the CO_2 enriched culture. The uptake was linearly correlated to photosynthesis in the first phase of growth, Figure 8. From the slopes of the regression lines, we calculated the percent decrease of the mineral uptakes : they was 38% for P, 29% for NO_3, 28% for NH_4 and 25% for K uptake.

In low density experiments similar results were obtained. The effect was reinforced for nitrate uptake which was relatively depressed in comparison with the carbon uptake, by a factor of 1.7 at day 22 after sowing. Results are in accordance with observations of the decrease of nitrogen content of dry matter previously reported by Madsen [37], Wong [13] and Allen et al [38]. Studies must be continued until the fructification

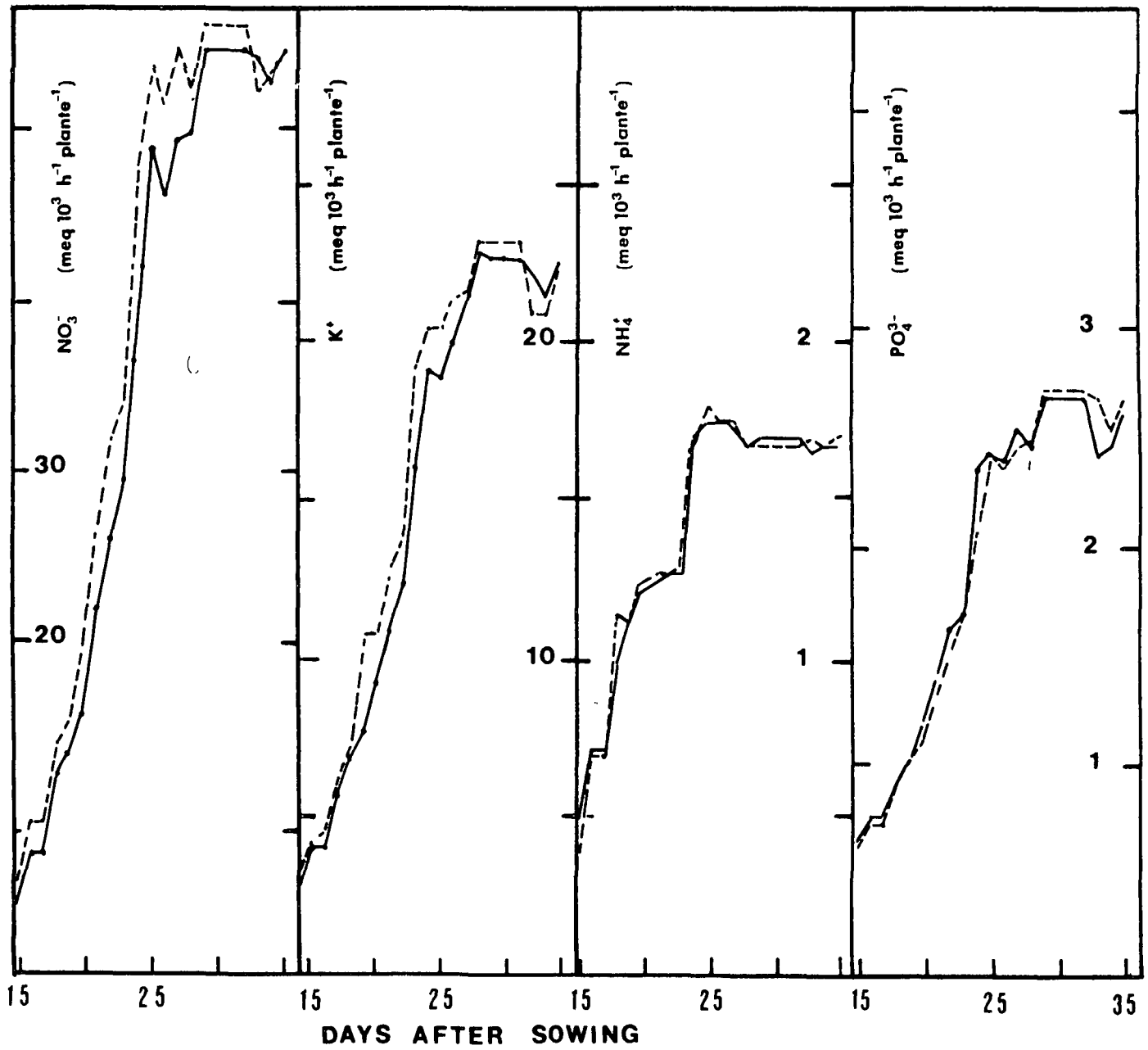

Figure 7 : Daily measurements of mineral uptakes obtained by quantitative monitoring of nutrient solution supply in two large samples of 100 to 60 plants of wheat cultivated with (———) 330 and (-----) 660 µl l^{-1} of CO_2. The figure is a reprinting of curves directly ploted by the computer which monitors chemical analysis.

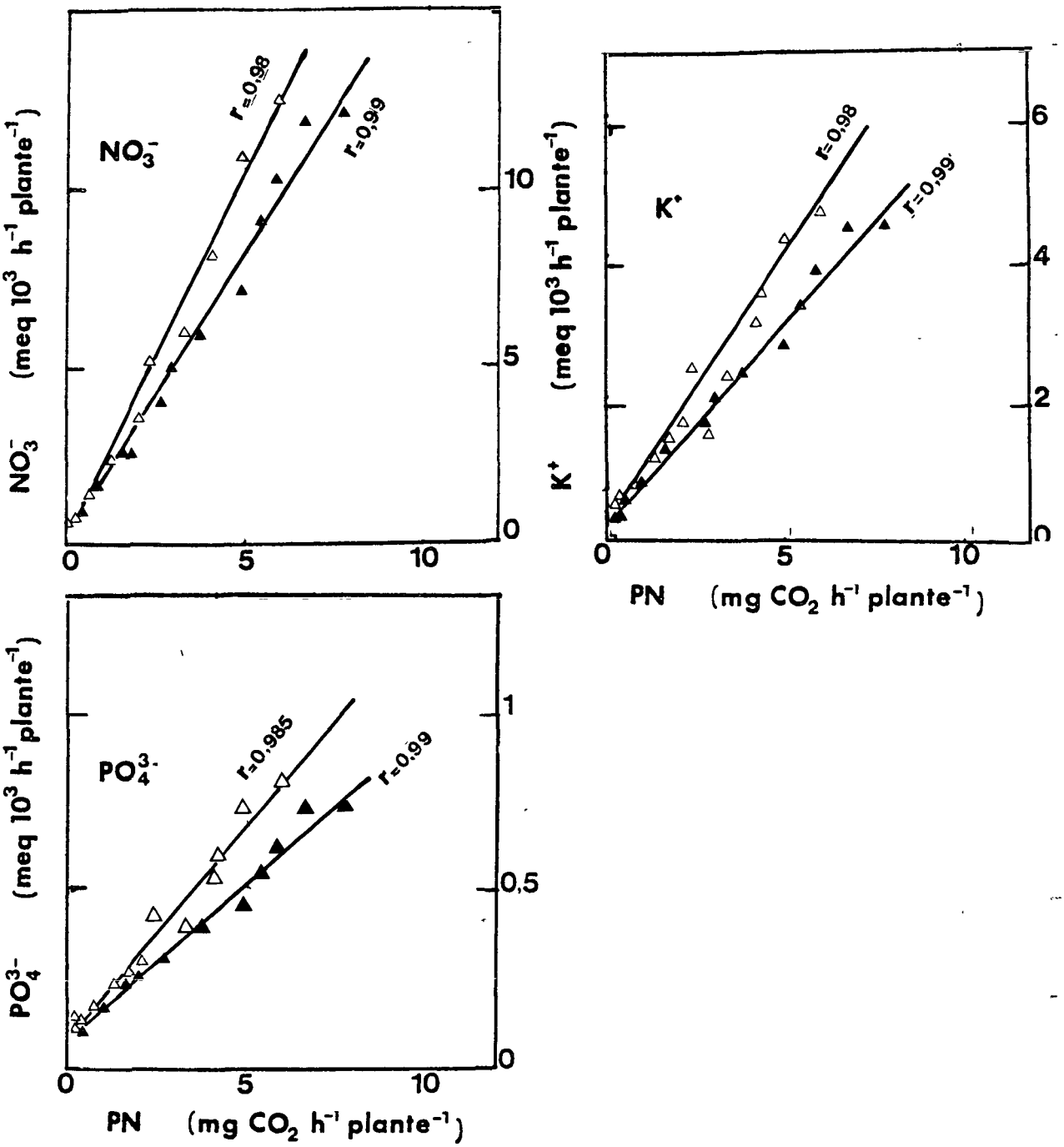

Figure 8 : Relationship between daily mineral uptakes and net daily photosynthesis for two cultures at high density with (△) 330 and (▲) 660 µl l^{-1} of CO_2.

stage and the harvest to know if such difference disturb the grain accumulation in wheat.

Conclusion

When simulating canopies planted in varied densities, we have been able to demonstrate that increase of dry matter production by enhancing CO_2 quickly becomes independant of increase of leaf area, especially above leaf area index of 2; dry matter gain results mainly from photosynthesis stimulation per unit of surface (primary CO_2 effet). When crop density is low (the plants remaining "alone" a longer time), the effects of increasing leaf surface (tillering, leaf elongation here, branching for other plants etc...) was noticeable and dry matter stimulation factor reached 1.65. This "area effect" decreased when canopy was closed in, as the effect of different surfaces no longer worked. The stimulation of photosynthesis reached to the "primary" CO_2 effect. The accumulation in dry matter which was fast during that phase made the original weight advantage more and more neglectible.

Comparison with short term measurements showed that first order long term effect of CO_2 in wheat is predictible with a short term experiment, from the effect of CO_2 on photosynthesis measured on reference sample.

We obtained a noticeable stimulation of dry matter for wheat (40 - 50 %) but it never reaches the very high figures we mentioned above taken from literature. These figures cannot be applied to plants in field conditions.

Unlike in some other research works we have not noticed any decrease in stimulation factor due to some negative feed-back, when the experiment is lengthened. So, if one represses for quite a long time photorespiration rate, it does not seems to have any negative consequence for wheat on the photosynthetic apparatus in our conditions. But this, only during the vegetative phase, when the nitrogen metabolism is not as active as in fructification stage.

We should note that transpiration and mineral uptakes in our culture under best conditions, were not changed notably in a doubling of CO_2 whereas carboxylation increases. These result in increase of about 50 % in water use efficiency and a decrease in mineral upake (-30 %) for

a given photosynthesis. Active research should be made in the consequences of the later point over the dry matter content especially in case of grain production.

During water limitations which reduces dry matter production by 20 to 40 %, in both canopies -with and without CO_2 enrichment -stimulation of matter production was maintained or even reinforced when strongly water limited. In that case water use efficiency reaches up to five times the standard figure.

These results emphasize the necessity to take into account simultaneously all the exchanges of the plant with its environment, in the shoot and it the root level as well.

We should like to make this point again, research without respect of density of planting cannot and must not be used in predictive models for field crops. Neither can they and must they be, in CELSS program whose density problem will be probably greater and crucial.

Acknowlegments - This work was partly supported by a grant of the Ministère de l'Industrie et de la Recherche (DGRST) in collaborattion with Pr. B. SAUGIER. We thank the staff of the Agro-Physiologie Laboratory at Cadarache : A. Daguenet, A. Gerbaud, D. Massimino, J. Massimino; J. Vivoli

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EFFECTS OF NO_3^-, NH_4^+, AND UREA ON EACH OTHER'S UPTAKE AND INCORPORATION

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The purpose of these studies is to determine the optimal use by wheat plants of the N sources expected from processing biological waste products, NO_3^-, NO_2^-, NH_4^+, and urea. Our approach is to determine the uptake and metabolic products of each N source (from single and multiple component solutions), inhibitory effects of each, feedback inhibition, and overall in vivo regulation of the rates of assimilation of each by wheat plants. Previously, we have determined the interactions of NO_3^-, NO_2^-, and NH_4^+ on each other's uptake and incorporation. This report deals with urea assimilation and some of its effects on NO_3^- and NH_4^+ assimilation which have been completed to date.

MATERIALS AND METHODS

Plant Material. Wheat (Triticum aestivum cv. Yecora Rojo) seedlings were grown hydroponically. Seeds were surface sterilized in sodium hypochlorite (5% v/v) for 15 min, rinsed with distilled water, and germinated at 25°C in aerated deionized water in the dark. After 24 h, the

germinated seeds were spread on a layer of cheesecloth supported on a stainless steel screen suspended about 1 cm above the surface of 1 l of aerated 0.2 mM $CaSO_4$ solution and placed in the dark at 25°C. After 7 days, the seedlings were transferred to aerated one-quarter-strength Hoagland solution lacking N (3) and placed in a controlled environmental growth chamber. Seedlings were grown under conditions of continuous light for 3 d at 25°C or for 3 weeks under a 16-h photoperiod at 25°/15°C light/dark temperature. Photon flux density at the seedling canopy was 400 $\mu Em^{-2}s^{-1}$, and RH was maintained at 60-65%. In some of the continuous light experiments, the seedlings were transferred after 2 days to nutrient solutions containing 1 mM ^{14}C-urea, NO_3^-, or NH_4^+ (preinduced seedlings).

$\underline{NH_4^+, NO_3^-, \text{ and Urea Uptake}}$. Uptake of NH_4^+, NO_3^-, and urea were determined by following their disappearance from the uptake solution with time as described previously in studies of NO_3^- uptake (1).

$\underline{\text{In Vivo Assimilation of Absorbed } NH_4^+, NO_3^-, \text{ and Urea}}$. $\underline{\text{In vivo}}$ assimilation of absorbed NH_4^+, NO_3^-, or urea were determined simultaneously along with uptake. The difference between the total amount of NH_4^+, NO_3^-, or urea absorbed and

that accumulated in the seedling, root, or shoot was considered to be assimilated *in vivo*.

NH_4^+, NO_3^-, and Urea Analysis. The tissue was ground with a chilled mortar and pestle in 5 volumes of deionized water and centrifuged at 30,000 x g for 15 min. NH_4^+ in plant extracts was determined by fluorimetric detection, following separation by HPLC (20 mM KH_2PO_4, pH 6.2) on a Whatman Partisil-10-SCX cation exchange column by post column derivatization with o-phthaldialdehyde (OPA) (5) determined spectrophotometrically at 210 nm following separation b HPLC on a Whatman Partisil-10-SAX anion exchange column (6). Urea was determined both spectrophotometrically at 189 nm and radiometrically. Urea was determined in plant extracts by counting plant extracts both before and after addition of excess urease. Free urea was determined by difference.

RESULTS

Analyses. This year we developed an automated HPLC assay for the uptake of urea along with NO_3^-, NO_2^-, and NH_4^+ by wheat seedlings from a full component nutrient solution utilizing a microcomputer-based system (Fig. 1). Except for the metabolic studies where a tracer is required, this

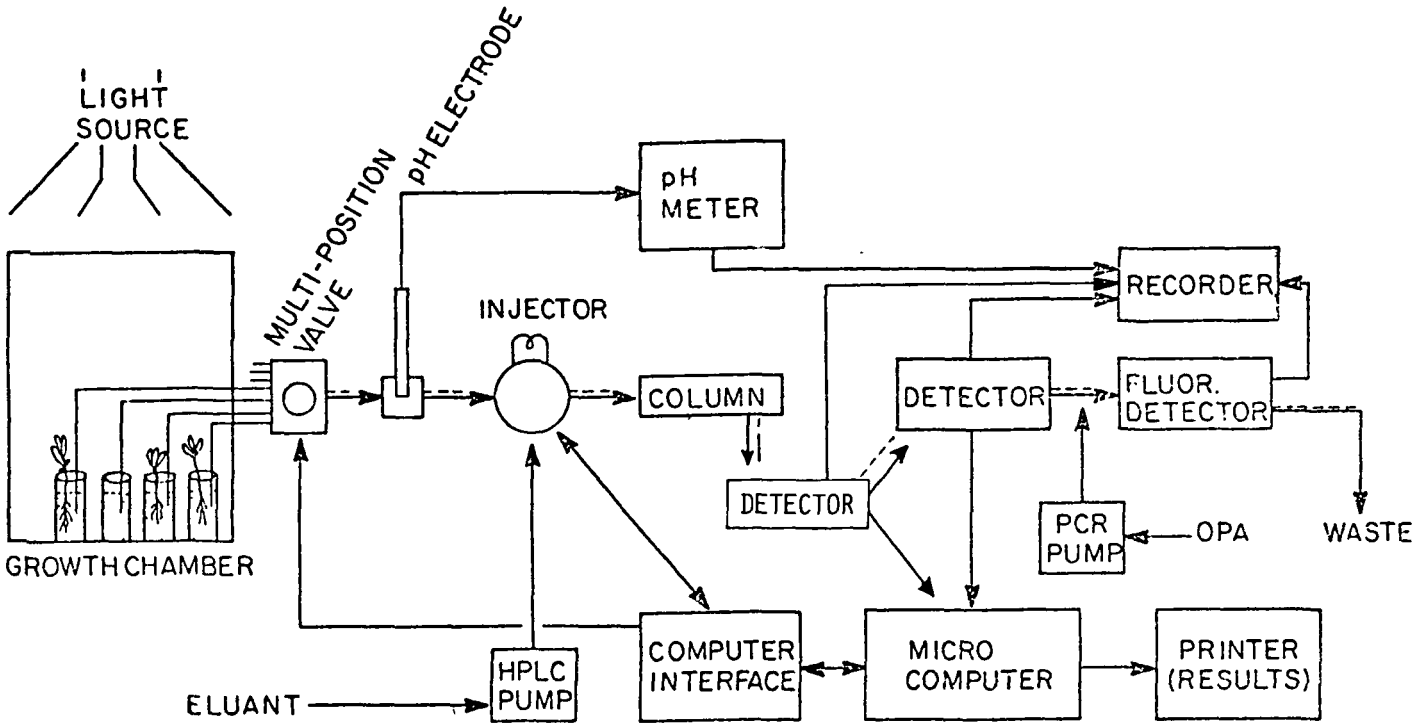

Figure 1. Flow diagram for determining uptake kinetics of N species by wheat seedlings. Detector 1 is a UV absorbance detector set at 189 nm for determination of urea. Detector 2 is a UV detector set at 210 nm for determination of NO_3^- and NO_2^-. Detector 3 is a fluorescence detector used for NH_4^+ determination.

removed the need to use ^{14}C-urea with its problems of contamination. This system was previously described in the analysis of NO_3^- and NO_2^-. The NH_4^+ assay has been improved by forming the OPA derivative in a flow system with detection by spectrofluorometry.

Uptake. Urea uptake was very slow in comparison to NO_3^- and NH_4^+ (Fig. 2) and showed a long lag before uptake began. The wheat seedlings were put in the presence of each of the above N compounds for 24 h to induce their transporters before the uptake studies were begun. The results shown are double the real rate of urea uptake since each urea molecule contains two N atoms. Of the three N compounds tested thus far, NH_4^+ uptake was the most rapid.

Assimilation. Figure 3 shows the concentration of urea, NH_4^+ and NO_3^- inside the plants. These concentrations are a function of uptake minus the amount further assimilated. Almost all of the urea absorbed by either roots or leaves is assimilated (Table I, Fig. 4).

Urea on NO_3^- and NH_4^+ Uptake. Urea facilitated the uptake of NO_3^- by wheat seedlings (Fig. 5), primarily because the induction of the NO_3^- transporter was much faster and a

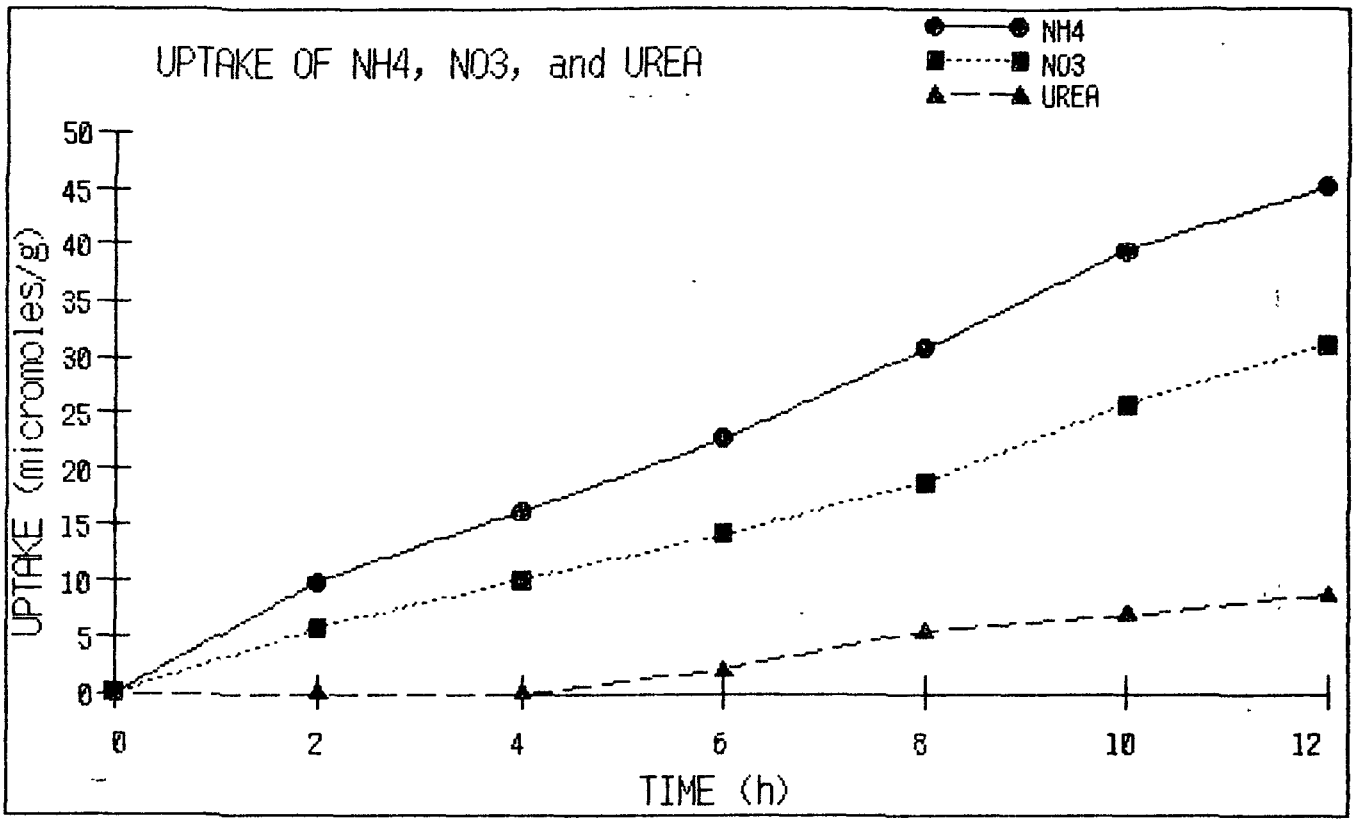

Figure 2. Comparison of urea, NO_3^-, and NH_4^+ uptake by pre-induced wheat seedlings. Seedlings were grown hydroponically in N-free solutions for 7 d in darkness followed by 2 d in continuous light. Groups of eight seedlings were transferred to pretreatment solutions containing 1 mM ^{14}C-urea, NO_3^-, or NH_4^+ in one-quarter strength Hoagland solution lacking N. After 24 h, the seedlings were transferred to 140 ml of fresh uptake solutions. Uptake of urea, NH_4^+, and NO_3^- were determined by sampling solutions every 2 h. Data is presented on a μmole N/g fresh weight basis.

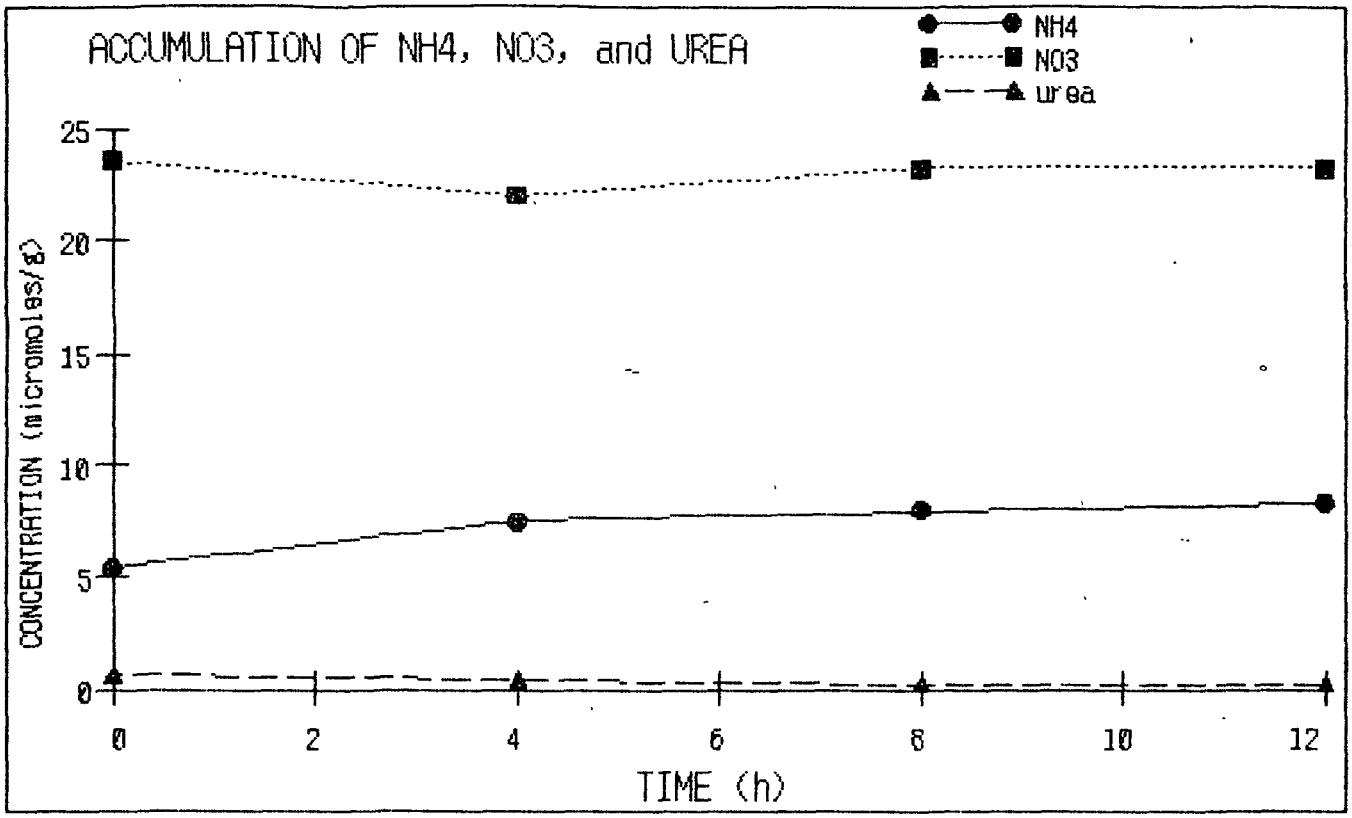

Figure 3. Accumulation of urea, NO_3^-, and NH_4^+ in preinduced wheat seedlings. Seedlings were grown as described in Fig. 2. NO_3^-, NH_4^+, and urea tissue levels were determined every 4 h as described in "Materials and Methods." Data is presented on a μmol/N g fresh weight basis.

Table I. Urea assimilation in wheat roots, leaves, and whole seedlings. Wheat seedlings were grown hydroponically for 3 d in continuous light as described in Fig. 2. Groups of 8 seedlings were separated into roots and shoots or used as whole seedlings. Roots and whole seedlings were placed in 40 ml of aerated uptake solutions containing 1 mM ^{14}C-urea. Shoots were placed base down in vials containing 10 mls of 5 mM ^{14}C-urea. Uptake and assimilation of urea were determined as described in "Materials and Methods" for one 6-h time point.

	Uptake	Assimilation	% Assimilation
	µmole N/gfw x 6 h		
Root	12	11.8	98
Shoot	10	9.9	99
Whole Seedling	11	10.8	98

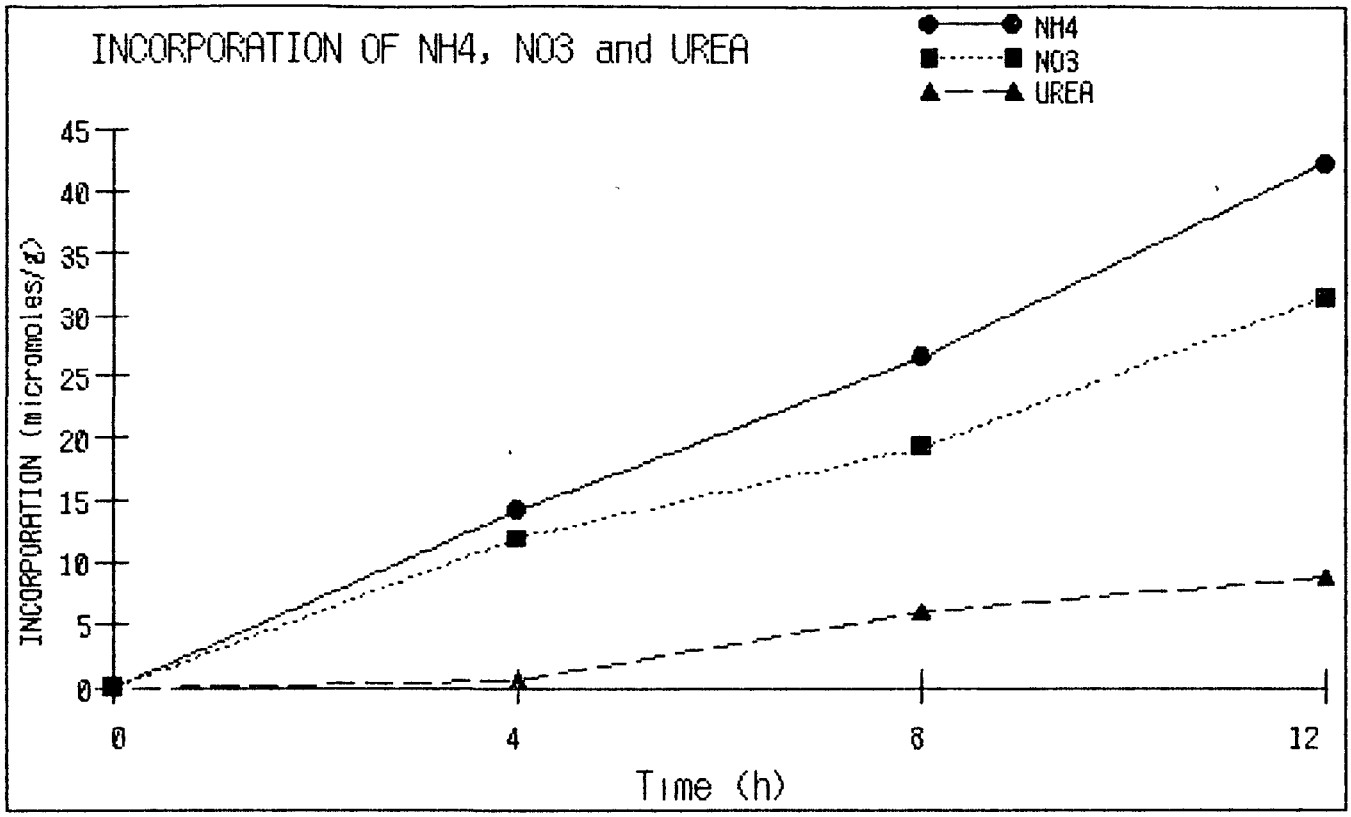

Figure 4. Assimilation of urea, NO_3^-, and NH_4^+ by preinduced wheat seedlings. Seedlings were grown as described in Fig. 2. N assimilation was determined as described in "Materials and Methods."

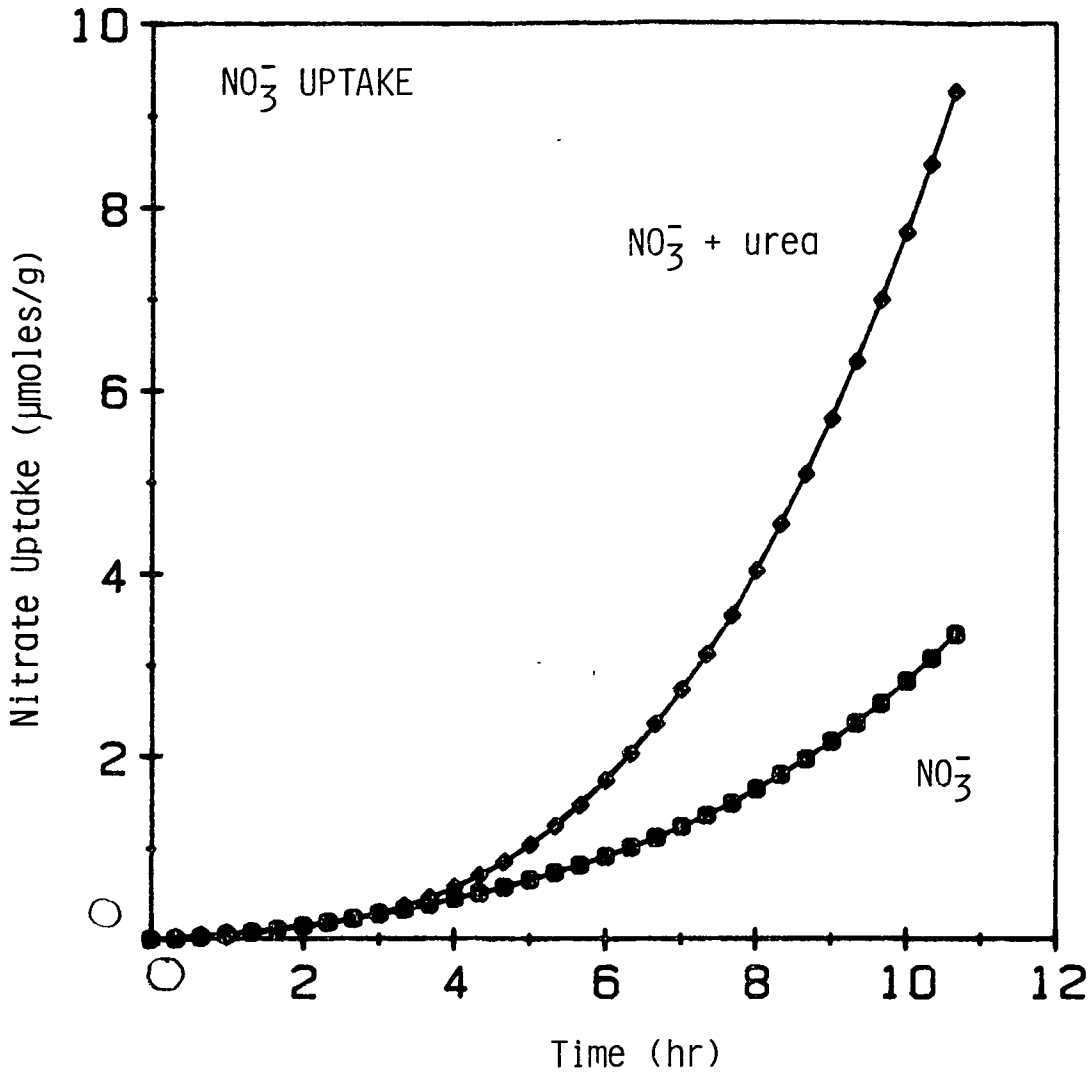

Figure 5. Effect of urea on NO_3^- uptake. Wheat seedlings were grown for 3 d in continuous light. Groups of 8 seedlings were transferred to 40 ml of uptake solutions containing 1 mM KNO_3 with and without 1 mM urea. Depletion of NO_3^- was monitored by sampling solutions every 20 min using an HPLC autosampling system.

greater induction occurred in the presence of urea. Urea had little effect on NH_4^+ uptake (Fig. 6).

Urea and NH_4^+ on NO_3^- Reduction in Wheat Leaves. NH_4^+ facilitated NO_3^- reduction while urea decreased NO_3^- reduction (Table II). Since this experiment was done on an equimolar basis, the urea supplied twice as much NH_4^+ as did the NH_4^+ treatment. We are at the stage now of supplying urea at half the concentration of NH_4^+ for a comparison on the basis of NH_4^+ concentration after assimilation of urea.

Growth of Wheat Plants in Urea. Wheat plants grew the least in urea compared to NO_3^- and NH_4^+ (Table III). As expected, the plants grew slightly better in NO_3^- than NH_4^+.

DISCUSSION

The automated analytical system described allows a precise estimate of the induction and activity of the transporters of the N compounds involved in the study. The importance of studying the inducibility of the transporters is to determine their stability throughout the growing season. Indications are that they decrease in both stability and activity as the plant roots age. If the transporters are under constant induction or turnover, as the

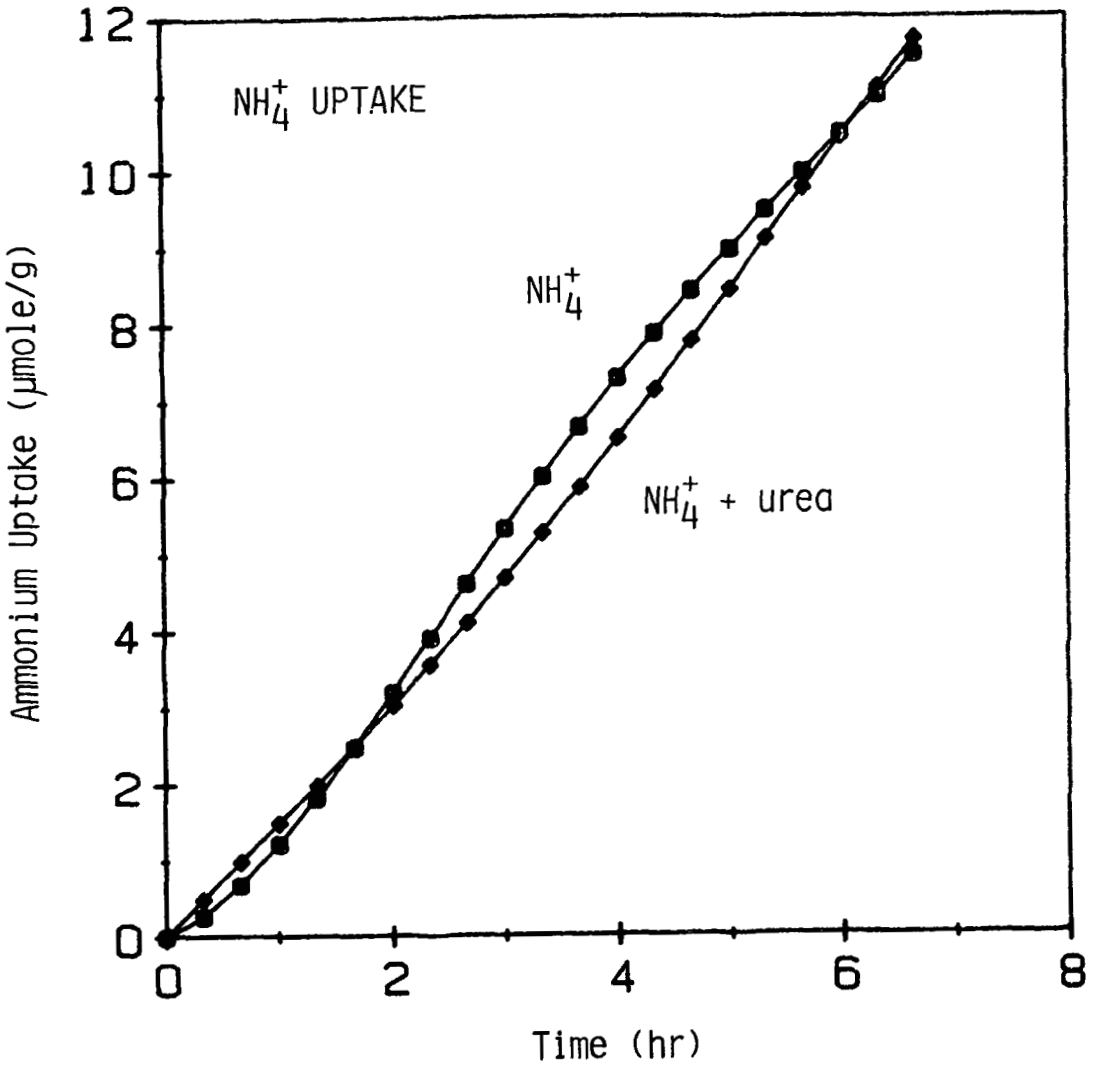

Figure 6. Effect of urea on NH_4^+ uptake. Wheat seedlings were grown for 3 d in continuous light. Groups of 8 seedlings were transferred to 40 ml of uptake solutions containing 1 mM NH_4^+ as $(NH_4)_2SO_4$ with and without 1 mM urea. Depletion of NH_4^+ was monitored by sampling solutions every 20 min using an HPLC autosampling system.

Table II. Effect of NH_4^+ and urea on NO_3^- reduction in wheat leaves in light. Wheat seedlings were grown for 3 d in continuous light. Leaves from groups of 8 seedlings were excised and placed base down in vials containing 10 ml of 5 mM NO_3^-, 5 mM NO_3^- + 5 mM NH_4^+ or 5 mM NO_3^- + 5 mM urea. Uptake and reduction were determined for a 24-h absorption period as described in "Materials and Methods."

Treatment	Uptake	Reduction	% Reduction
	µmol/gfw x 24 h		
NO_3^-	90	65.7	73
NO_3^- + urea	92	48.8	53
NO_3^- + NH_4^+	91	79.2	87

Table III. Effect of urea, NH_4^+, and NO_3^- on the growth of wheat plants. Wheat seedlings were grown hydroponically in N-free solutions for 7 d in continuous darkness, followed by 2 d under a 16/8-h light/dark regimen. Groups of 5 seedlings were then transferred to 5 l of nutrient solutions containing one-quarter strength Hoagland solution lacking N. Nitrogen was supplied as 1 mM KNO_3, 1 mM NH_4HCO_3 or 1 mM urea. Solutions were changed daily. Root and shoot weights were determined at the end of the 3-week experimental period.

N Source	Root	Shoot	Whole Plant
		weight (g)	
NO_3^-	7.2	12.4	19.6
NH_4^+	6.7	11.4	18.1
Urea	4.3	8.0	12.3

plant ages, it may lose or develop decreased ability to maintain their presence in the plasmalemma. Do the transporters disappear when the inducing N compounds are depleted from the nutrient solution? Can they be reinduced when the inducing compound is again added to the nutrient solution? Are they stabilized when the inducing compound is always present?

It has been shown previously that the NO_3^- and NO_2^- transporters are induced by the presence of their substrates (2, 4). Indications are that the urea transporter is induced and that the induction requires quite a long time (over 2 days) (Fig. 2). Work is continuing to verify this observation.

Uptake of urea is extremely slow when compared to NO_3^- or NH_4^+ (Fig. 2), which seems to result in decreased growth (Table III) with urea as the only source of N. Once urea is taken up, it is very efficiently assimilated; therefore, uptake seems to be the rate-limiting step to its utilization by wheat seedlings. When the four N species used in our studies are fed to roots, NH_4^+, urea, and NO_2^- are assimilated almost totally in the roots while NO_3^- is assimilated primarily in wheat leaves. Thus, NO_3^- seems to be the only one of the four whose reduction may be facilitated more directly by

photosynthetically derived electrons. Urea decreased NO_3^- reduction in leaves (Table III), however, since urea is mainly assimilated in roots, it will likely have little effect on NO_3^- assimilation on a whole-plant basis. This remains to be determined.

At equimolar concentrations, urea facilitated NO_3^- uptake (Fig. 5). The increased uptake was largely due to decreasing the time required for induction of the NO_3^- transporter and increasing the amount of transporter induced. Urea had little effect on NH_4^+ uptake (Fig. 6).

Future studies:

1. Determine if presence of urea induces a urea transporter or if the transporter is constitutive.
2. Determine the stability of the transporters as wheat ages.
3. Complete the studies showing the interactions of urea, NH_4^+, NO_3^-, and NO_2^- on each other's induction, uptake, and further assimilation in roots and intact seedlings.
4. Complete growth studies as a function of the assimilation of the four mixed N sources at differing concentrations.

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STUDIES ON MAXIMUM YIELD OF WHEAT FOR THE CONTROLLED ENVIRONMENTS OF SPACE

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The economic feasibility of using food-producing crop plants in a Controlled-Environment Life-Support System (CELSS) will ultimately depend on the energy and area (or volume) required to provide the nutritional requirements for each person. Energy and area requirements are, to some extent, inversely related; that is, an increased energy input results in a decreased area requirement and vice versa.

A major goal of our research effort is to determine the controlled-environment food-production efficiency of wheat per unit area, per unit time, and per unit energy input. We have studied wheat for the following reasons:

(1) Its grain can be processed into a wide variety of food products that can supply a major portion of dietary carbohydrates and protein. Certain other crops (e.g., lettuce) can supply only a small portion of dietary calories before toxic levels of vitamins or secondary products are consumed.

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(2) Wheat, rice, and maize (in that order) are the major food crops of the world. Much is known about wheat physiology, and this knowledge can be rapidly adapted to new environments.

(3) Much is also known about wheat genetics, so it is possible to quickly select and breed new cultivars for a new environment.

(4) Wheat forms flowers in response to long days (i.e., it is a long-day plant) and grows well in continuous light, which results in a maximum use efficiency per unit mass of the lighting system. Short-day crop plants such as rice have an obligate requirement for a dark period (about 8 to 12 hours, depending on species and cultivar) before they initiate seed production. Tomatoes cannot grow under continuous light, which causes their leaves to become chlorotic (yellow) and eventually die.

(5) The vertical leaf orientation allows wheat to efficiently absorb high levels of photosynthetically active radiation (400 to 700 nm) and convert this energy into a high grain yield per unit area. Crop plants with horizontal leaves efficiently absorb low levels of radiation but generally are less efficient when radiation levels are high (Gar-

dner et. al. 1985; Leopold and Kriedemann, 1975).

Based on our productivity findings from the past few years, we can now calculate the size and energy requirements for each person in a CELSS. First, however, it is useful to compare our findings with theoretically achievable limits, and then with more realistic potentially achievable productivities.

THE THEORETICAL MINIMUM SIZE OF A CELSS

At the CO_2 concentrations present in the earth's atmosphere, species with C_4 photosynthesis (e.g., maize, sugarcane) are often more efficient than species with C_3 photosynthesis, which include wheat and most crops (summary in Salisbury and Ross, 1985). At elevated CO_2 levels, however, C_3 plants are significantly more efficient than C_4 plants. C_3 crop plants are therefore an excellent choice for a CELSS or a lunar station, where CO_2 levels are expected to be elevated. From the stoichiometry of electron transport in photosynthesis and a proton requirement of three for ATP synthesis (Handgarter and Good, 1982), a theoretical minimum of 9 moles of photons are required to fix 1 mole of CO_2 into carbohydrates in C_3 species. This is known as a quantum requirement of 9 or a quantum yield (reciprocal of quantum requirement) of 0.111. The theoretical quantum requirement

of C_4 species is 15 (quantum yield of 0.067).

At the whole plant level, however, some energy is required for nitrate reduction, some is lost to fluorescence, some is absorbed by nonphotosynthetic pigments and reradiated as heat, and some is used in the respiratory synthesis of complex molecules, so the best achievable quantum yield is 0.083 (quantum requirement = 12). This has been achieved only at low irradiance levels and in the absence of photorespiration (Ehleringer and Pearcy, 1983; Osborne and Garrett, 1983). This quantum yield is close to the conversion efficiencies achieved with algae.

If we could achieve a continuous quantum yield of 0.083 from a photosynthetic photon flux (PPF) of 1000 μmol m^{-2} s^{-1} (about one half full sunlight at the earth's surface), we could theoretically produce 216 g m^{-2} d^{-1} of carbohydrates. (Calculations: 1000 μmol m^{-2} s^{-1} X 86,400 s d^{-1} X 0.083 = 7.2 mol CO_2 m^{-2} d^{-1} X 30 g (mol CH_2O)$^{-1}$ = **216 g m^{-2} d^{-1}.**) Assuming 4 kcalories per gram of carbohydrates and a requirement of 2600 kcal person^{-1} day^{-1}, then we can calculate (216 g m^{-2} d^{-1} X 4 kcal g^{-1} divided into 2600 kcal person^{-1}) a minimum continuous-food-production area of only **3 m^2 per person.**

THEORETICAL ENERGY REQUIREMENTS

McCree (1972) calculates that 5 μmols of photons s^{-1} produced by high-pressure sodium lamps in the photosynthetic part of the spectrum (400 to 700 nm) represent almost exactly one watt of energy. Thus, if high-pressure sodium lamps can be made 40 percent efficient at producing photosynthetic energy (efficiency of 37.6% is noted below), an input of 500 W m^{-2} could produce 1000 μmol m^{-2} s^{-1}. If 3 m^2 were required per person, the energy input could be as low as **1.5 kW per person.** This is the highest possible efficiency that could be achieved by any plant species.

POTENTIALLY ACHIEVABLE SIZE AND ENERGY REQUIREMENTS

This anaylsis of theoretical size and energy requirements is highly simplified. The following factors reduce the achievable productivity of higher plants below the theoretical maximum.

1. Light Absorption.

Plant leaves never absorb all the incident radiation. We have found that, under ideal conditions, 5 percent of the photosynthetically active radiation is reflected, and 1 percent is transmitted, even by a dense canopy with vertical leaves. It is unlikely that absorbed energy will ever exceed 95 percent of incident photosynthetic energy.

A more significant absorption problem occurs during the early stages of growth when small plants do not cover the surface area and consequently cannot intercept all the incident irradiation. We grow wheat at densities up to 1500 plants m^{-2} (6.7 cm^2 $plant^{-1}$, 2.6 cm between plants). This is 3 to 6 times normal planting densities in the field, but plant leaves absorb only 50 percent of the irradiance when they are 14 days old and 90 percent when 18 days old. After day 18, light interception continues to be excellent until harvest at day 60. The germinating seeds do not require light until emergence on day 3, but absorption efficiency is low from day 3 to about day 18. In our current system, this loss is about 20 percent of the total area and energy required to grow the crop. A mechanical system or transplanting to alter plant spacing during early growth (so plants are moved apart as they mature) could eliminate some of this loss. Such systems are being used in commercial controlled-environment food production.

Increasing plant densities even beyond those we have used would also improve early interception of radiation before day 30, but this approach has two problems, one engineering and one biological: (1) We have not yet designed a system to support ultra high densities of plants above a hydroponic root-zone environment. (2) Interplant competi-

tion for light quickly becomes considerable at high plant densities. Little is known about the effects of this low-light stress on young plants, but field studies indicate that high densities decrease seed yield before total plant growth is reduced (see discussion in Gardner et al., 1985).

2. Harvest Index.

The most significant limitation to food production is that not all the biomass produced by the plants is edible. The edible divided by total biomass (both dry) is called the harvest index. A lettuce crop has about 80 percent edible leaves and 20 percent inedible stem and roots. Potatoes can have a harvest index of edible tubers of 80 percent of the total biomass, and wheat can reach 60 percent edible grain on a dry-mass basis. Under the best conditions, there is a 20 to 40 percent loss from inedible plant parts.

Many authors have suggested crops with edible roots, leaves, and reproductive structures. Sweet potato and sugar beet are examples. In most cases, however, only the young leaves are edible, although it is the mature tubers, roots, fruits, or seeds of such plants that are normally harvested. Unusual food crops should be considered for a CELSS, but claims of high productivity and high harvest index often cannot be substantiated.

3. Digestible energy per unit edible biomass.

When the energy content of oven dry wheat grain is determined by combustion in a bomb calorimeter, values as high as 3.94 kcal g^{-1} are obtained, but the digestible energy is only about 3.7 kcal (Table I). This relationship also holds for other food commodities.

Table I

DIGESTIBLE ENERGY CONTENT OF HARD RED SPRING WHEAT GRAIN

	As Harvested (%)	Oven Dry %	kcal g^{-1}		Kcal g^{-1} In Bomb Calorimeter
Carbohydrates	66.8	76.8	x 4	=	3.07
Protein	14.0	16.1	x 4	=	0.64
Fat	2.2	2.5	x 9	=	0.23
Fiber	2.3	2.6	0		--
Ash	1.7	2.0	0		--
Moisture	13.0	0.0			--
	100.0	100.0			3.94

Net Usable Digestible Energy Varies from about 90 to 95% of the 3.94 Kcal g^{-1} (See FAO publication 24. 1970. Amino Acid content of Foods and Biological Data on Proteins.)

Assuming 94% digestibility:

$$\begin{array}{r} 3.94 \\ \times\ .94 \\ \hline 3.70 \end{array} \text{ kcal } g^{-1}$$

$$\begin{array}{r} \times\ 4.18 \\ \hline 15.0 \end{array} \text{ joules Kcal}$$

$$\text{joules } g^{-1}$$

4. Energy conversion.

High-pressure sodium lamps produce 376 W of energy between 400 and 700 nm per 1000 watts input power. This makes them 37.6% efficient (personal communication: Chris Mpelkas, Sylvania Test and Measurements Lab, Danvers, MA). Their output, however, must be reflected down onto the plants. The best reflectors are about 90 percent efficient. This makes the overall efficiency of the system 33.8 percent. Efficiencies of 27.4 percent have been achieved on a commercial scale. The Phytofarm in Dekalb, Illinois, has an energy input of 219 W m^{-2} from high-pressure sodium lamps and a photon output of 300 µmol m^{-2} s^{-1} or 60 W m^{-2} of photosynthetic irradiance (Maynard Bates, personal communication).

5. Respiration.

Respiration provides the energy to synthesize complex molecules from the initial products of photosynthesis. It occurs at the same rate in wheat leaves in both the light and the dark (Azcon-Bieto and Osmond, 1983). When wheat is grown in continuous irradiation, all the respiratory synthesis of compounds must occur in the light. This respiratory evolution of CO_2 decreases the quantum yield and may be part of the reason a quantum yield of 0.111 has not been achieved in plant leaves.

Assuming that all respiration takes place in the light and that we can continuously achieve a quantum yield of 0.083, there is still a loss of fixed carbon to root system respiration. This loss is proportional to the biomass of the root system. Field grown crop plants typically invest 20 to 30% of their biomass in roots, but our hydroponically grown wheat plants invest only about 10% of total biomass in roots.

Carbon dioxide evolution during photosynthesis from "dark" respiration is taken into account in our calculations by assuming a quantum yield of 0.083 rather than 0.111.

Considering these factors, the potentially achievable size and energy requirements can be calculated as follows:

Theoretical (with 1000 µmol m^{-2} s^{-1} PPF)	216 g m^{-2} d^{-1}
95% light absorption over life cycle	205 g m^{-2} d^{-1}
80% harvest index	164 g m^{-2} d^{-1}
Multiplied by 3.7 kcal g^{-1} (94% digestible)	607 kcal m^{-2}
Assume 3000 kcal per person per day:	**5 m^2 person^{-1}**

Energy requirement:

1000 µmol m^{-2} s^{-1} =	200 W m^{-2}
200 W m^{-2} divided by 0.338 efficiency =	592 W m^{-2}
592 W m^{-2} x 5 m^2 = 3552 W person^{-1} =	**3.6 kW person^{-1}**

These theoretical efficiencies will be very difficult to achieve, but they represent a long term goal. They will be extremely difficult to achieve with a crop plant (like strawberries) that is chosen for its asthetic qualities and flavor rather than for its productivity. Nonetheless, research will need to be done on all species grown in a CELSS to optimize their edible productivity.

CURRENTLY ACHIEVABLE PRODUCTIVITIES WITH WHEAT

During the past year, after spending much time on designing and building research chambers to create optimum environmental conditions for studies on wheat productivity, we have obtained reproducible production data that can be used to estimate the size of a CELSS. We have been highly successful in converting photosynthetic irradiance into biomass but less successful in converting total biomass into edible yield.

Quantum Yield and Short-Term Photosynthesis

We measure short-term rates of carbon fixation in wheat canopies with a gas exchange system that includes a pressurized growth chamber. A canopy of 0.8 m^2 is grown in this chamber with the roots in a sealed, recirculating, hydroponic system. A photosynthetic photon flux of 1000 µmol m^{-2} s^{-1} in an atmosphere enriched to 1700 µmol CO_2 (mol air)$^{-1}$ has re-

sulted in photosynthetic rates as high as 58 μmol m^{-2} s^{-1} of carbon dioxide absorbed per unit horizontal area. Ninety-four percent of the photon flux was absorbed, resulting in a quantum requirement of 16.2. This can be compared to the theoretically achievable quantum requirement of 9 and potentially achievable requirement of 12.

If we subtract for root respiration and multiply by the photoperiod each day, this figure can be converted into a daily growth rate. Root biomass in our hydroponic systems is typically only 10 percent of the total (20 to 30% in the field). Subtracting this estimated 10 percent respiratory loss and assuming continuous light, this photosynthetic rate should result in a growth rate of 135 g m^{-2} d^{-1}. This compares well with the theoretically achievable growth rate of 205 g m^{-2} d^{-1} (at 95% light absorption).

Measured Growth Rates.

We measure actual growth rates at weekly intervals by removing a 0.2 m^2 section of plants (about 200 plants in a rigid support), blotting the roots dry, weighing the section, and returning it to the hydroponic solution. A few plants are destructively harvested and dried to determine percent dry mass, from which dry-mass growth rates can be calculated. We have measured growth rates of 875 g m^{-2} $week^{-1}$ or 125

g m^{-2} d^{-1}. This growth rate serves as a validation of the short-term photosynthesis measurements. Unfortunately, it takes about 22 days for a group of plants to reach this growth rate, and the rate gradually decreases as the plants mature. These factors combine to make a best-achievable growth rate of 89 g m^{-2} d^{-1} averaged over a 60-day life cycle.

The production of 89 g m^{-2} d^{-1} total biomass is truly remarkable by conventional agricultural standards. Typical field productivities are less than 10 g m^{-2} d^{-1}, and 20 g m^{-2} d^{-1} is exceptional (Table II). Wheat is obviously stressed even in the best field conditions. The stress factors could be low carbon dioxide and/or low light, neither of which would be economical to change in the field.

Yield and Harvest Index.

These high growth rates are the good news. The bad news is that we have not yet been able to cause wheat growing at high rates to partition a normal percentage (40 to 50%) of its total biomass into edible grain. A crop producing 89 g m^{-2} d^{-1} should have a grain yield of 35 to 45 g m^{-2} d^{-1}; our best yield has been only 21.4 g m^{-2} d^{-1}. At 21.4 g m^{-2} d^{-1}, **30 m^2 and 15 kW person^{-1}** would be required. This is our currently achievable productivity. Table III indicates some of the growth parameters associated with this yield.

Table II

A COMPARISON OF WHEAT PRODUCTIVITIES IN DIFFERENT ENVIRONMENTS

	Absolute Seed Yield $g\ m^{-2}$	Life Cycle days	Yield Per Day $g\ m^{-2}\ d^{-1}$	Total Biomass $g\ m^{-2}$	Biomass Per Day $g\ m^{-2}\ d^{-1}$	Harvest Index %
Field Production	300-700	90-130	3-7	700-1800	7-18	45
World Record in Field*	1450	120	12.1	3200	27	45
CELSS Project	1200	56	21.4	5000	89	24
Russian Study**	1000	56	17.9	2860	51	35

*Winter Wheat (sown in autumn; actually in the field about 270 days).

**Achieved with 50% of CELSS project energy input (Gitel'zon, 1977).

Table III

HIGHEST CURRENTLY ACHIEVABLE YIELDS

Cultivar	Total Biomass $g\ m^{-2}\ d^{-1}$	Edible Biomass $g\ m^{-2}\ d^{-1}$	Days In Growth Chamber	Harvest Index %	Plants Per m^2
Fremont	89.0	21.4	56	24.0	715
Anza	69.0	21.4	60	31.0	450

Plants harvested from a 400 x 500-mm experimental plot area (0.2 m^2) guard rows not included in calculation.

The problem of low harvest index is caused by low seed number per head. A comparison of our yield components with field production data clearly indicates the problem, as shown in Table IV.

Table IV

YIELDS IN CONTROLLED ENVIRONMENTS AND IN THE FIELD

	Life cycle days	Seed yield g m^{-2}	Harvest index percent	Heads per m^2	Seeds per head	Mass per seed mg
Controlled environment	60	1300	25%	3000	15	29
High yield from field	120	800	45%	800	30	33

Continuous light and a constant high temperature (27°C) are principle factors responsible for shortening the life cycle from 120 to 60 days. These same two factors may also be responsible for the low seed number per head. Low seed number per head is the combined result of two factors: (1) fewer spikelets formed on the head (spike) during the floral induction phase (days 4 to 13) and (2) poor pollination during anthesis (days 25 to 35).

There is evidence that long photoperiods result in the production of fewer spikelets per spike during floral induc-

tion (Rawson, 1970; Lucas, 1972). David Bubenheim, a graduate student, is now conducting studies to quantify the effect of photoperiods as long as 24 hours on rate of reproductive development in wheat. The most significant of his preliminary findings are as follows (Bubenheim and Salisbury, 1985):

(1) The length of the vegetative phase is extremely short in continuous light. In one cultivar, reproductive development began 48 hours after germination.

(2) The length of the initiation phase, during which spikelet number is determined, is inversely proportional to photoperiod between 12 and 24 hours. A short initiation phase results in fewer spikelets per spike (head) and fewer potential seeds per head.

(3) Development of daylength-insensitive cultivars (from Mexico) is faster in long photoperiods during the vegetative and initiation phases, but the stem elongation phase is greatly retarded by long photoperiods. These two effects offset each other, making the cultivars appear insensitive to photoperiod.

A second problem is poor pollination and seed set in existing florets. Wheat is self-pollinated, and the anthers

(male flower parts) either do not form or do not grow normally after pollination in our conditions. Microscopic examination has indicated that pollen is often aborted before it accumulates starch, while embryo sac (female flower part) development is completely normal.

We are just beginning to study the problem. Our environmental conditions are very different from those in which wheat evolved, so many environmentally induced factors could be contributing to cause poor seed set. Two parameters, however, are associated with pollen sterility: nutrient (especially boron) deficiency and high temperatures. Boron and calcium are passively absorbed and translocated in the transpiration stream to foliar plant parts. The foliar concentrations of these elements are therefore determined by their concentration in the root zone and by the ratio of transpiration to photosynthesis. In the field this ratio is typically about 200 to 250 grams of water transpired per gram of biomass produced. The high CO_2 levels in our controlled environments cause photosynthesis to increase, but, by causing stomates to close, transpiration is decreased. Transpiration/photosynthesis ratios can be as low as 50:1. Calcium and boron concentrations in leaf tissue are decreased accordingly.

We compensate for the reduction by using 2.5 times normal calcium and boron concentrations in the initial hydroponic solutions of young plants. This change results in tissue concentrations of these elements that are similar to those found in field-grown wheat leaves, but the nutrient imbalance caused by high CO_2 may not be completely alleviated in all plant parts. Foliar applications of boron have increased fruit set in prune trees that had adequate foliar levels (Hanson and Breen, 1985). The amount of calcium and boron is typically low in wheat heads, and it is especially low in developing heads just previous to emergence from the leaf sheath. One of the classic symptoms of boron deficiency is poor pollen viability (Kirkby and Mengel, 1983). We therefore analyzed the concentrations of 13 elements in wheat heads before and after emergence in 12, 18, and 24-hour photoperiods with 700 µmol mol^{-1} CO_2 environment. Photoperiod had little effect on the amount of any element in the heads; indeed, with the exception of calcium, magnesium, and boron there was almost no effect of developmental stage on nutrient composition. The changes in these 3 elements are shown in Table V.

The concentration of these elements is low in postemergence heads and extremely low before emergence, but these data do not necessarily indicate a deficiency of these ele-

ments. We are currently analyzing additional normal head samples to determine adequate levels of these elements.

Table V

CONCENTRATIONS OF THREE ELEMENTS IN WHEAT HEADS

(Mean ± STD. Deviation)

Stage of Head Development	Ca	Mg	B
	----------%----------		µg g-1
Preemergence (Day 27)	0.05±.02	0.09±.02	4.6±1.6
Postemergence (Day 50)	0.34±.10	0.27±.03	9.3±1.2

High temperatures are also associated with pollen sterility in wheat. A recent report indicates that cool night temperatures might be especially beneficial (Carlson and Williams, 1985). We have begun studies in shorter photoperiods, but we have not yet provided particularly cool night temperatures (27oC day, 25oC night). It may be important to provide cool dark periods during the week of anthesis.

Within the next year, we expect to solve the harvest index barrier to high yields. Finding the solution could rapidly increase our currently achievable yields. Meanwhile, we have examined several cultural, environmental, and genetic approaches to yield optimization.

The Effect of CO_2 Concentration on Growth and Yield.

Table VI summarizes data from the first of two carbon-dioxide-enrichment studies conducted in the fall of 1984. The trial compared responses of dwarf (50-cm tall) and semi-dwarf (80-cm tall) wheat cultivars grown at three CO_2 concentrations: 350 µmol mol^{-1} (ambient), 700 µmol mol^{-1}, and 1800 µmol mol^{-1}.

Table VI

THE EFFECT OF CO_2 CONCENTRATION ON TOTAL AND EDIBLE BIOMASS OF WHEAT*

CO_2 Concentration µmol mol^{-1}	Vegetative Fresh Mass On Day 28 g	Total Biomass** g m^{-2} d^{-1}	Edible Biomass** g m^{-2} d^{-1}	Harvest Index %	Root Mass %	Seeds Per Head
350	1218	47.5	15.8	33.3	7.5	18.7
700	1966	70.0	19.8	28.3	8.8	16.0
1800	2066	56.1	13.6	24.2	15.3	12.0

*Each value represents the mean of 4 replicate plots of 0.2 m^2 each.

**These values do not include a 7-day germination period in the greenhouse.

Table VII shows responses of three of these cultivars plus another one in the greenhouse. Although sunlight in the greenhouse was supplemented with light from high-pressure-sodium lamps (24 h d^{-1}), the total irradiance was lower than

Table VII Part A

RESPONSE OF CERTAIN WHEAT CULTIVARS TO
ENRICHED CARBON DIOXIDE IN THE ATMOSPHERE
(October, 1984, trial)

Location CO_2 Concentration ($\mu mol\ mol^{-1}$)	Cultivar	Total Grain Yield $g\ m^{-2}$	Days to Maturity	Grain Yield Per Day (oven dry) $g\ m^{-2}\ d^{-1}$	Plants m^2	Heads per m^2	Seeds per Head	Harvest Index %
Greenhouse	Fremont	681	59	11.5	450	1729	17.3	39
700	Fremont*	701	60	11.7	450	1318	24.3	36
	PCYT 20	584	60	9.7	450	1187	18.6	41
	Y. ROJO	427	56	7.6	450	1654	13.7	36
Chamber 2	FMT	842	57	14.8	450	1290	23.3	32
350	FMT HD**	931	57	16.3	715	1786	20.5	34
	Anza	957	60	15.9	450	1730	17.8	35
	PCYT 20	528	60	8.8	450	1540	13.1	32
Chamber 3	FMT	1079	63	17.1	450	2355	16.5	25
700	FMT HD	1196	63	18.9	715	2893	15.5	24
	Anza	1284	67	19.2	450	2019	18.1	31
	PCYT 20	1041	67	15.5	450	2262	14.0	33
Chamber 1	FMT	850	59	14.4	450	1832	18.8	37
1800	FMT HD	942	59	15.9	715	2125	17.3	26
	Sonoita	594	59	10.1	450	2495	7.2	20
	PCYT 20	492	63	7.8	450	3224	4.7	14

*Two replicate plots of Fremont (FMT) were grown in the greenhouse; the differences between the plots represent experimental error.

**HD = high density (715 plants m^{-2}; all other treatments included 450 plants m^{-2})

Table VII Part B

Location; CO_2 Concentration µmol mol^{-1}	Cultivar	Root Mass %***	Plant Height cm	Vegetative Growth (grams fresh mass) Day 17	Day 23	Day 28
Greenhouse	Fremont*	12	73	260	612	1035
	Fremont*	11	65	196	508	936
	PCYT 20	12	50	175	450	823
	Y. ROJO	9	45	249	627	956
Chamber 2	FMT	7	65	226	601	1161
350	FMT HD**	8	70	387	899	1526
	ANZA	9	79	215	649	1287
	PCYT 20	6	47	227	506	896
Chamber 3	FMT	11	81	372	1338	2223
700	FMT HD	8	82	573	1798	2901
	Anza	8	85	304	865	1581
	PCYT 20	8	58	221	586	1160
Chamber 1	FMT	20	77	423	1186	1912
1800	FMT HD	13	77	-	1710	2435
	Sonoita	13	50	573	1421	2153
	PCYT 20	15	57	329	1004	1762

*Two replicate plots of Fremont (FMT) were grown in the greenhouse; the differences between the plots represent experimental error.

**HD = high density

***Figures for root mass indicate percent of total plant dry mass.

it was in the three chambers. It varied during the life cycle from 400 to 1000 µmol m^{-2} s^{-1}, whereas irradiance in the three chambers was held at 1000 µmol m^{-2} s^{-1}.

For the trials, a new system was devised to hold the plants. Instead of styrofoam lids with foam plug inserts, U-

shaped metal bars were placed side by side and held together with long bolts. Seedlings are held in closed-cell foam rubber (weather stripping) between the bars. Transplanting is faster in this system, and it allows us to use much higher plant populations. The distance between rows is fixed at either 30 or 38 mm, but the spacing within rows can be varied to achieve a wide range of plant densities. The dimensions of the lids are 400 x 500 mm.

A second replicate trial confirmed the findings of the first carbon-dioxide-enrichment study. In both studies total plant biomass was considerably increased by CO_2 enrichment, but grain yield and harvest index were higher at 700 µmol mol^{-1} CO_2 than at 1800 µmol mol^{-1} CO_2. These data suggest that the highest CO_2 concentration may be too high for maximum yields, but if high CO_2 is inducing a secondary effect, such as nutrient deficiency, then adjustment of other environmental parameters would lead to increasing yields even at the highest CO_2 level. The stomatal closure induced by high CO_2 greatly reduces transpiration, but reducing humidity increases transpiration, which may increase nutrient uptake and ultimate yield. We have preliminary data suggesting that reduced humidity increases nutrient uptake in high CO_2 conditions. Reducing transpiration with high carbon dioxide and high humidity might save energy (that required to evaporate

water) in a CELSS.

Table VIII quantifies the effect of CO_2 enrichment on the ratio of grams of water given off in transpiration to grams carbohydrates fixed in photosynthesis.

Table VIII

TRANSPIRATION/PHOTOSYNTHESIS RATIO
(g H_2O/g CH_2O)

CO_2 µmol mol^{-1}	PPF 900 µmol m^{-2} s^{-1}	PPF 2100 µmol m^{-2} s^{-1}
350	177	90
700	122	65
1800	60	49

Typical field values are 200 to 250

These transpiration rates are very low, but they do not appear to be an inherent problem. Transpiration is important for cooling plant leaves, but even the lowest transpiration rates provide adequate cooling in our conditions, and low transpiration rates still provide ample purified water for a life support system. The limiting factor may be nutrient uptake, but nutrients could be applied as foliar sprays.

THE EFFECT OF INCREASED PHOTOSYNTHETIC PHOTON FLUXES ON PHOTOSYNTHESIS

Plant growth research in controlled environments is typically conducted at photosynthetic photon fluxes between 300 and 1000 μmol m^{-2} s^{-1}. Photosynthesis of single leaves of C_3 crops light saturate in this range, but leaves in crop canopies complete for available light so that dense communities of vigorous crop plants often do not light saturate even in full sunlight (2,000 μmol m^{-2} s^{-1}). If increased energy inputs lead to increased productivity, the size of a CELSS food production facility can be reduced accordingly. It is not yet known whether energy or volume will be the more limiting constraint.

Growth and yield over a range of energy inputs should provide valuable design information. We have obtained short term (7-day) photosynthetic responses to PPF levels of up to 2,100 μmol m^{-2} s^{-1}. These measurements were made in an open gas exchange system that utilizes a sealed growth chamber as the assimilation chamber. The design of this system is discussed in Sestak, Catsky, and Jarvis (1971). Photosynthetic calculations are described in Nobel (1983). These results are shown in Figures 1 and 2. It is apparent that photosynthetic rate in a CO_2 enriched environment is light limited even in the highest naturally occuring light levels.

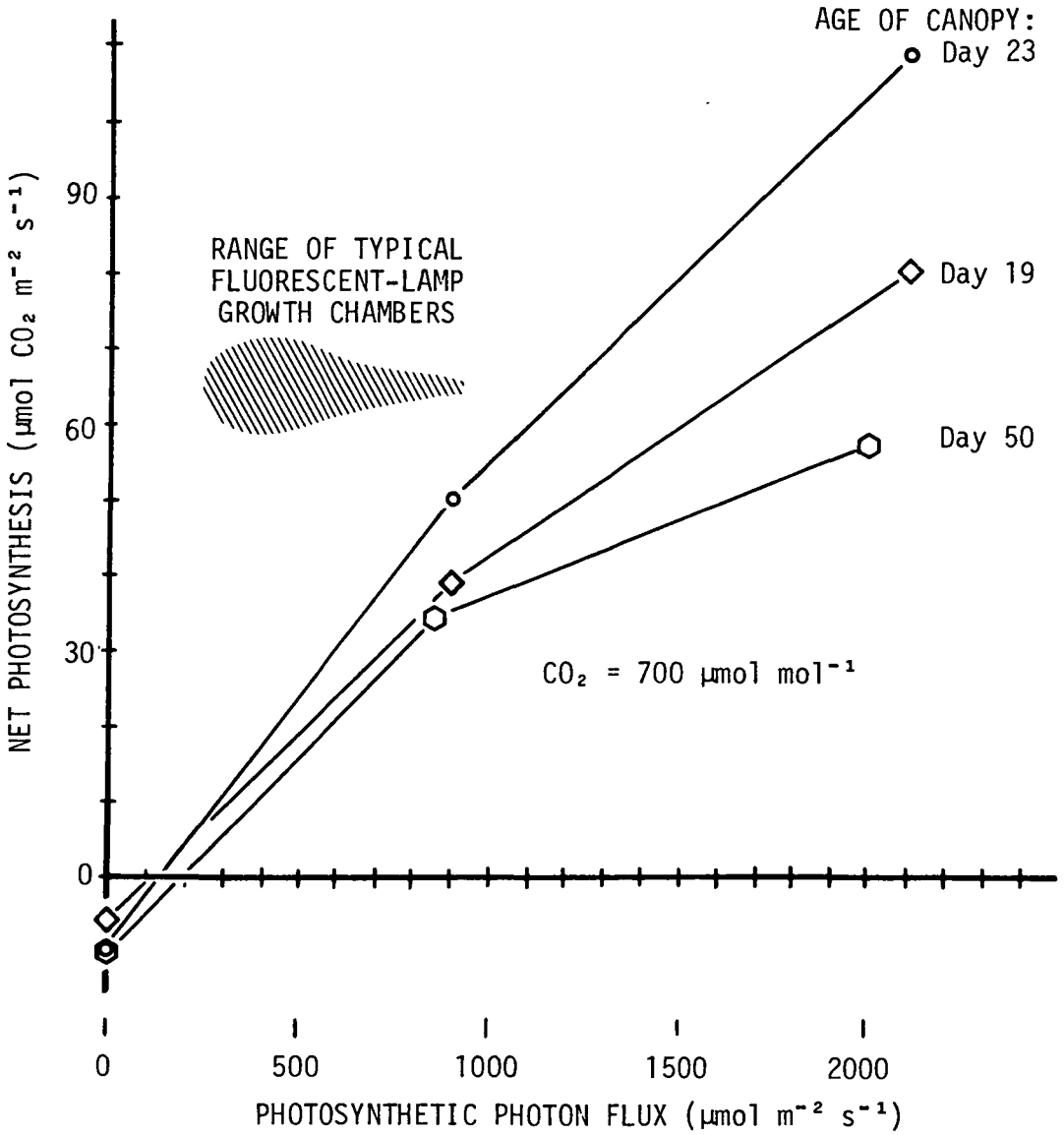

Figure 1 Net photosynthesis as a function of irradiance level. Note that photosynthesis of a dense canopy increases with increasing light at least up to the level of sunlight. Note also that most growth chambers in use today (which use fluorescent plus incandescent lamps; PPF about 250 to 900 µmol m^{-2} s^{-1}) are just above the light compensation point. At such a light level, doubling the irradiance more than doubles net photosynthesis.

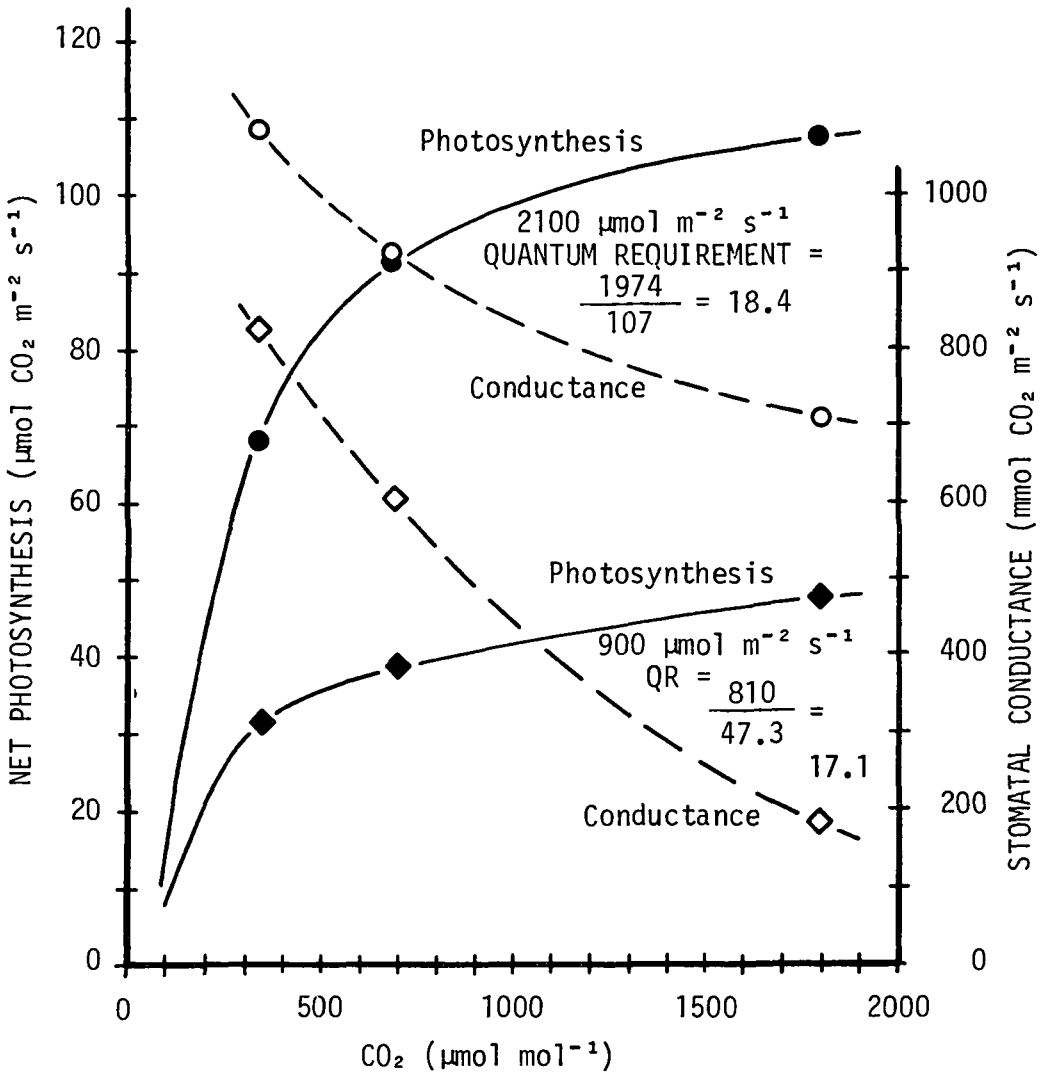

Figure 2 Net photosynthesis and stomatal conductance as a function of carbon-dioxide concentration at two irradiance (PPF) levels. Note that conductance decreases sharply as CO_2 increases, indicating stomatal closure. Photosynthesis increases with increasing CO_2, but the increase with increasing light is much more striking. Higher light levels also induce stomatal opening, increasing conductance. (Curves for photosynthesis are extrapolated to an approximate CO_2 compensation point of 100

We studied the longer term effects of high irradiance by exposing a plant canopy to 2100 μmol m^{-2} s^{-1} for 24 h d^{-1} for 7 days. No change in CO_2 uptake was observed during this period. We also examined the initial photosynthetic rate after a 4-hour dark period, during which assimilates could be unloaded and exported from the leaves. Again, no change in net photosynthesis was observed. These results suggest that feedback inhibition of photosynthesis is not a serious problem even in continuous light. If these rapid growth rates can be translated into edible productivity, the option of substituting energy for volume over a wide range will be available to CELSS system designers.

The Effect of Planting Density on Growth and Yield

As discussed earlier, poor light absorption during early growth considerably reduces energy efficiency. When ample nutrients, water, and oxygen are available in the root zone, high plant populations should lead to enhanced early growth, which might also lead to higher yields. This hypothesis was tested using our newly designed plant support lids (discussed earlier). A very rapid circulation rate in our hydroponic solution minimizes any root zone deficiencies so that competition for light is the primary limiting factor. Table IX shows the results of an initial study with a 24 h d^{-1} photoperiod and with a PPF of 700 μmol m^{-2} s^{-1}.

Table IX

THE EFFECT OF PLANTING DENSITY ON GROWTH AND YIELD

Plant Density m^{-2}	Vegetative Fresh Mass Day 22	Total Biomass At Harvest g m^{-2} d^{-1}	Edible Biomass At Harvest g m^{-2} d^{-1}	Heads m^{-2}
380	368	39.2	9.1	2000
680	585	41.0	7.6	2800
1060	722	38.2	9.2	2900
1320	855	48.8	15.7	3700
High Intensity Field Production:				
400	—	20.0	8.0	800

The growth parameters at each density represent one 400 x 500 mm block of plants with the guard rows removed.

Higher plant densities are clearly associated with increased vegetative fresh mass for the first 22 days of growth. Growth parameters at harvest show an inconsistent, but generally positive response to high densities. Additional tests need to be conducted.

BREEDING A CULTIVAR FOR CELSS

The production of multiple heads per plant, known as tillering, is a desirable phenomena in field production, but it decreases plant uniformity and harvest index and is thus

undesirable in controlled environments where uniculm cultivars can be planted at high densities.

We now have cultivars that do not tiller in the field. In our enriched controlled environment conditions, however, they tiller almost as much as control plants. Elevated levels of nutrients (especially nitrogen), water, CO_2, and light are all known to promote tillering. The combination of these factors has prevented us from testing the uniculm concept in our studies. Theory continues to predict that uniculms would perform better in high production environments, but the germplasm is not yet available.

We have had considerable success in breeding ultradwarf (less than 50-cm) cultivars. The results of a replicated yield trial are shown in Table X.

Yields tend to be slightly less than our highest yielding dwarf lines, Sonoita, Yecora Rojo, and PCYT 20, but plant height has been reduced considerably. Consequently, yield per m^3 has been improved. Harvest index should also be increased, but the immediate advantage is that ultradwarf lines are much easier to work with in confined, controlled environmental areas. The difficulties of providing a uniform environment increase exponentially with plant height.

Table X

YIELD PERFORMANCE OF BEST
ULTRADWARF ADVANCED BREEDING LINES

Cultivar	Height cm	Yield g m^{-2}	Yield g m^{-3}
39-1-1	29	49.2	170
40-1-3	34	49.2	145
44-3-2	44	54.8	125
50-1-1	37	53.6	145
Commercial full dwarf wheats			
Sonoita	44	51.0	116
Yecora Rojo	46	65.9	143
PCYT 20	52	69.9	134
Olesen's Dwarf*	27	40.5	150
Semidwarf wheat	90		
Full size wheat	150		

*Olesen's Dwarf was the source of dwarfing genes for these breeding lines.

Last spring we selected 121 lines from the CIMMYT germplasm collection in Mexico. One of these cultivars is less than 20 cm tall, and several others show great promise for genetic yield enhancement. We continue to evaluate new material in both the field and in a hydroponic greenhouse section that is enriched with CO_2 and supplemented with high-pressure-sodium light.

FOOD PRODUCTION IN SIMULATED MICROGRAVITY

Microgravity may be more important to high productivity than any other environmental parameter, yet we have no information on the long term responses of crop plants to microgravity conditions, and only a few long term studies have been attempted with plants in the simulated microgravity environment of clinostats.

The gravity compensation environment of clinostats may or may not be an accurate simulation of true weightlessness for plants. This question will remain unanswered until more data from spaceflight experiments are available for comparison. In the meantime, we are attempting to obtain information on long-term crop-plant responses to weightlessness by using clinostat simulation.

Eliminating inadvertent environmental stress while providing gravity compensation on a clinostat is a challenging task, but minimizing stress is especially important with crop plants. Crop plants have been bred for rapid growth when environmental conditions are favorable, and they are generally more sensitive to stress than noncrop species. Our objective has been to design a clinostat to minimize mechanical stress and provide the favorable environmental conditions that allow crop plants to express their full growth

potential.

A clinostat was constructed to provide high intensity radiation from "above" the plants at all times. Thirty-four pots were placed on the inside of a 1.4-m diameter cylinder so that plant tops were directed at the axis of rotation. The cylinder is divided vertically (right angles to the axis of rotation) with plywood painted white. One 400-W metal halide lamp (Sylvania Super Metal Arc) is placed at the axis of rotation on each side of the plywood. The lamps were protected by glass cylinders, which were ventilated with small fans so that radiant heating of plant leaves could be minimized. The rotation rate of the clinostat is controlled by a variable speed, DC electric motor, which is connected to the central axis by a gear-reduction chain drive. The rotation rate for all experiments was 0.6 rpm, which resulted in a gravitational acceleration of 2.8×10^{-4} g at the outside edge of the clinostat. This is well below the threshold g response for shoots and at or below the threshold response for roots.

A capillary-tube, drip-irrigation system was used to provide nutrient solution as the plants moved past the bottom on each revolution. Fiberglass window screen was stretched over the top of each pot to hold the soilless media (1:1:1 ratio, peat:perlite:vermiculite) in place during rotation.

Table XI

WHEAT YIELD COMPONENTS AS
AFFECTED BY MICROGRAVITY SIMULATION

	total bio-mass g	total seed mass g	harvest index %	heads per plant	seeds per head	mass per seed mg
CONTROL	9.4	3.8	40	10	18.4	20.4
CLINOSTAT	9.8	2.6	27	7	20.0	19.6

Results are preliminary, but Table XI summarizes our findings with wheat. We can now draw the following conclusions (Bugbee and Salisbury, 1985).

(1) Peas, soybeans, and wheat are capable of producing viable seed while being continuously grown on a clinostat.

(2) Plant growth rates are not necessarily affected by clinostating.

(3) Structural support of foliar plant parts on clinostats may be helpful, although the contact with the support could itself be stressful (Jaffe, 1976).

(4) Irradiation from "above" the plants may be necessary to eliminate a photocompensation effect.

(5) Plants on this clinostat do not consistently bend in the direction of rotation (Hoshizaki and Hamner, 1982; Tibbitts and Hertzberg, 1978).

PLANT TISSUE CULTURE FOR RAPID REGENERATION OF HYBRID WHEAT

(John G. Carman)

Our primary goal in the tissue culture of wheat is to consistently regenerate 1000 or more uniform, high-yielding hybrid plants from the callus initiated from either a 4-mm slice of young inflorescence tissue or an individual wheat embryo. We are optimistic that we can reach this goal within two years. Our short range objectives are to:

(1) Identify wheat cultivars that show promise for tissue culture regimes.
(2) Optimize the culture medium for callus induction, somatic (asexual) embryoid proliferation, and somatic embryoid germination on agar-solidified medium.
(3) Optimize culture conditions and growth regulator concentrations for somatic embryoid proliferation in cell suspension cultures.

Our accomplishments to date include:

(1) Identification of a winter wheat that infrequently produces, in callus culture, somatic embryoids that are identical in appearance and germination properties to sexually formed embryos.
(2) Identification of two spring wheat cultivars that

undergo extensive somatic embryogenesis in callus culture. Unfortunately, the embryoids produced are not as well formed as those produced in the winter wheat cultivar.

(3) Continued optimization of culture medium variables such that a 2-fold increase has been realized for some wheat cultivars in frequency of somatic embryoid formation over former culture media used for wheat tissue culture as currently reported in the literature.

As we develop suspension culture systems for wheat tissue cultures, we hope to be able to achieve our goal of 1000 plants per explant. Recently, such systems that have been developed for rice have yielded far greater numbers of plants, with theoretical projections of 126,000 plants per explant within a six-month culture regime.

CONCLUSIONS

The data in this paper provide many reasons to be optimistic about enhancing our current productivities. We now have the research equipment and instrumentation to study physiological responses to unusual environmental conditions, and we especially look forward to data from CELSS experiments in space. We are also interested in the problems created by nearly complete closure of a ground-based facility.

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UTILIZATION OF POTATOES IN CELSS: PRODUCTIVITY AND GROWING SYSTEMS

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The potato plant (Solanum tuberosum L.) has been recognized as one of the basic food crops that should be studied for use in NASA's Controlled Ecological Life Support System (CELSS) (Tibbitts and Alford, 1982). It offers high yields per unit area and time, with most of this production in the form of highly digestible carbohydrate (Smith, 1977). Potatoes, like wheat and rice, are particularly useful in human diets because of their nutritional versatility and ease of processing and preparation.

Potatoes can be grown effectively with stem cuttings from plants maintained in sterile culture, thereby eliminating most insect and disease problems and greatly increasing the plant to plant uniformity for research investigations. For our studies, plantlets 10 cm high are grown from the stem cuttings in test tubes on agar under sterile culture and then transplanted into containers of non-sterile peat vermiculite or other media. Upon transplanting, these small plants grow rapidly and after 28 days can attain a diameter exceeding 40 cms. The plants develop vigorous side branches that produces a spreading, vine like plant. As plants enlarge, the leaves orient to

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effectively intercept all of the incoming irradiation. A canopy with 3 leaf layers per unit ground area (i.e. a leaf area index of 3) absorbs over 95% of the incident radiation (Figure 1).

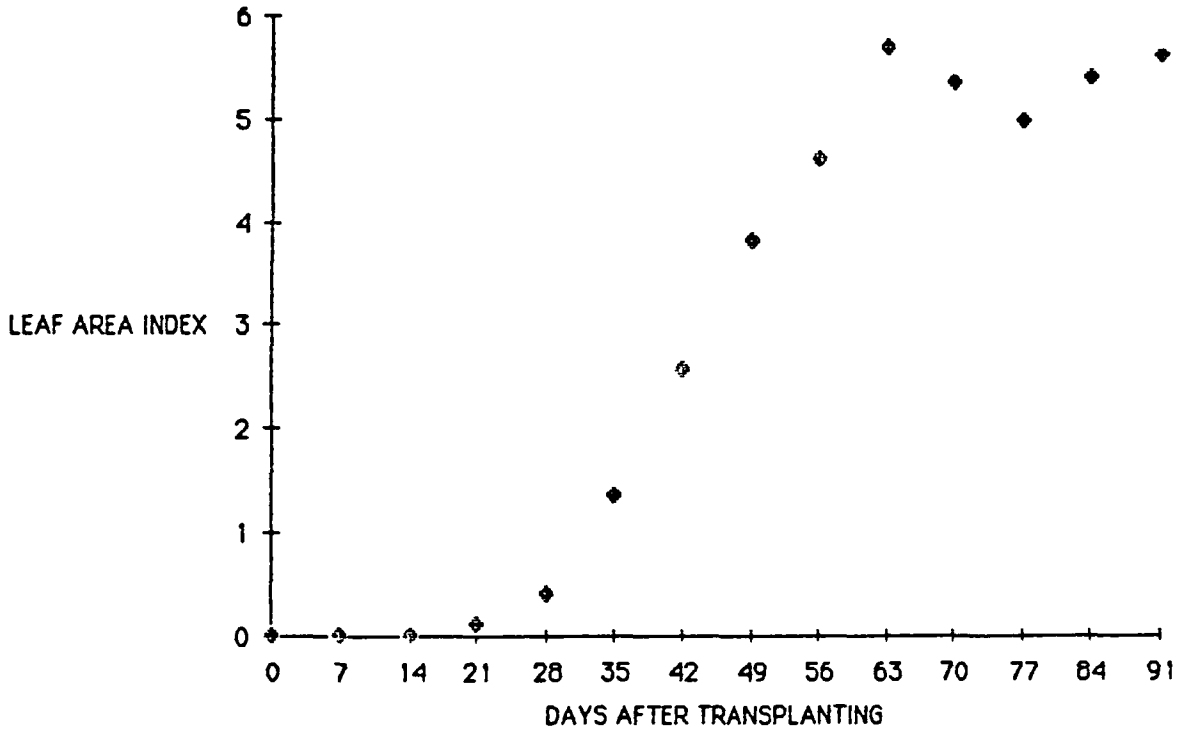

Figure 1. Leaf area index of potato plants spaced 45 cm apart and grown with 400 μmol $s^{-1}m^{-2}$ irradiance with 12-hr photoperiod at 20°C and 70% RH.

Tubers form on stolons (underground horizontal stems) that extend from the buried portion of the stem. In some cultivars stolons are very short and tubers form almost sessile to the stem, whereas in other cultivars stolons will extend 0.3 to 0.5 m from the stem and develop tubers along their entire length. Tuber production is regulated to some

extent by the volume of media available for root and stolon extension; for example, 38-liter containers provided greater tuber production than 19-liter containers (Figure 2).

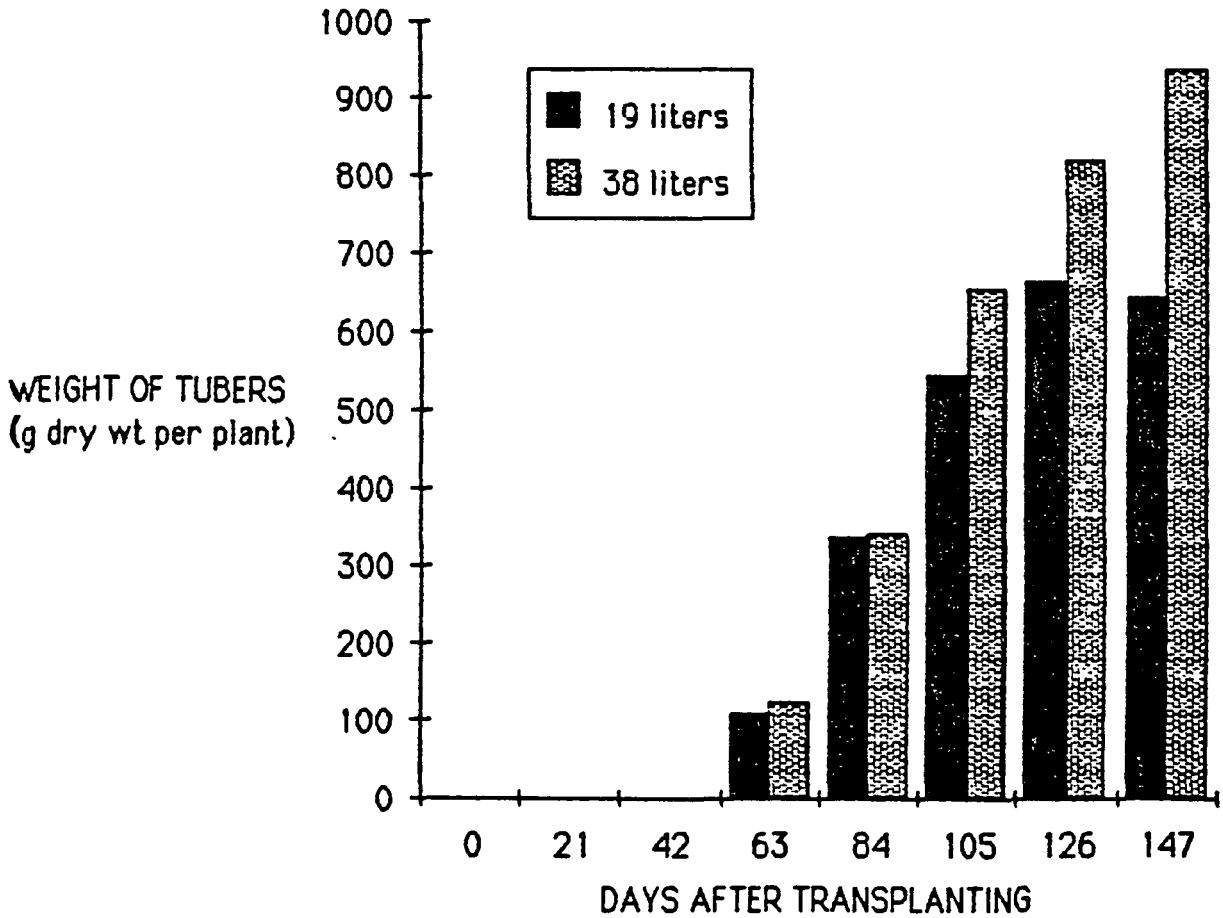

Figure 2. Effect of container size on tuber weight of potatoes at different stages of development. Plants grown with 400 μmol $s^{-1}m^{-2}$ irradiance with 24-hr photoperiod at 16°C and 70% RH.

Plants were larger in the 38-liter containers and accumulated significantly more tubers during final weeks of growth between 105 and 147 days after transplanting.

Tuber production is controlled to a significant extent

by many different environmental factors including photoperiod, irradiation level and temperature (Bodlaender, 1963). These factors affect both the total dry matter accumulated per unit time and the allocation of this dry matter between tubers and other vegetative organs of the plant.

Photoperiod has long been recognized to regulate tuberization, with short photoperiods encouraging tuberization and long photoperiods slowing tuberization (Bodlaender, 1963). Our experiments have shown that long photoperiods do decrease the proportion of photosynthates partitioned to the tubers but with increases in total irradiance, tuber production can be obtained despite suppressing effects of the long photoperiods. Thus if irradiance levels are high enough, tuber production can be very large under long photoperiods. A 15-week study of tuber formation of potatoes maintained with 12, 16 and 20-hr of irradiance at 400 μmol $s^{-1}m^{-2}$ and 20^{o}C temperature demonstrated that the highest yields occurred under 20-hr irradiance; with the least under 16 hr (Table 1). However,

Table 1. Tuber production of 15-week-old 'Superior' potatoes grown under different irradiance durations at 400 μmol $s^{-1}m^{-2}$ level and 20°C temperature.

Irradiance period (hrs)	Tuber dry weight per plant (g)	Tuber percentage of total biomass
12	257	61
16	224	43
20	293	43

of the three treatments, the 12 hr plants had the greatest proportion of dry weight in the tubers, i.e. the highest "harvest index". In another study of only 6 weeks duration, we found that continuous irradiance at a level of 400 μmol $s^{-1}m^{-2}$ also produced more tubers than 12 h of irradiance at this same level (Table 2). However continuous irradiance at a level of 200 μ mol s^{-1} m^{-2} produced essentially no tubers.

Table 2. Tuber and total biomass production of 'Norland' potatoes grown for 6 weeks under different irradiance treatments and 16°C temperature.

Irradiance		Dry weight per plant (g)	
Duration (hrs)	Level (μmol $s^{-1}m^{-2}$)	Total biomass	Tubers
12	400	53	12.2
24	400	125	19.6
24	200	80	0.4

These data also demonstrated that the efficiency of total dry mass accumulation can be as good or better under continuous irradiance than under 12 hr irradiance, for a doubling of total irradiance with 24 hr irradiance more than doubled the dry matter obtained with 12 hr irradiance. This study also demonstrated that a 200 μmol s^{-1} m^{-2} irradiation level is more photosynthetically effective than 400 μmol $s^{-1}m^{-2}$, for the plants under low irradiance had more total dry weight per unit of irradiance.

It is of particular interest that certain cultivars do not tolerate long photoperiods. This is seen in the stunting of cv. 'Kennebec' plants grown under continuous light. These plants developed to be only 25 cm high and 25 cm in diameter after 8 weeks of growth compared to a healthy cv. 'Norland' plants of which were more than 50 cm high and 100 cm in diameter after 8 weeks of growth. Also, 'Kennebec' was found to be partially stunted under 20 hr irradiation and even slightly stunted under 16 hr irradiation. Another cultivar, 'Superior' was found to be severely stunted under 24 hr irradiance periods but not affected by 20 and 16 hr irradiance. 'Norland' and 'Russet Burbank' cultivars showed no apparent stunting under continuous irradiance.

Temperature has been found to be a very significant

variable for regulation of the proportion of dry matter allocated to the tubers and for regulation of the length of the plant stems (Bodlaender, 1963). Reducing the temperature increases the proportion of dry matter allocated to tubers and reduces the stem length. In studies comparing growth at 12°C, 16°C, 20°C, 24°C, and 28°C, maximum tuber production was obtained at 16° (Table 3). Plants at 16° had

Table 3. Development of 'Norland' potatoes grown for 8 wks under different temperature levels.

Temperature (°C)	Tubers (g dry wt) per plant	Total biomass (g dry wt) per plant	Tubers (%)	Stem length (cm)
12	73	123	59	18
16	123	209	59	37
20	96	186	51	48
24	2	151	1	74
28	0	116	-	94

less shoot growth than plants at higher temperatures, indicating that a greater percentage of the photosynthetic production was allocated to the tubers. At high temperatures nearly all dry matter production was allocated to the shoots and essentially none to tuber formation. At 12°C, total growth was slowed but a high proportion of the

photosynthates were allocated to tuber formation. The reduction in stem length with cool temperatures is particularly significant with regard to minimizing total plant volume in a CELSS plant growth facility.

We are now involved in study of the interacting effects of carbon dioxide concentration, temperature and irradiance level on tuber formation. We have established a split plot matrix study involving the following levels and combinations:

Carbon Dioxide ($\mu l\ l^{-1}$):	400, 1000, 1600
Temperature (^{o}C):	16, 20, 24
Irradiance ($\mu mol\ s^{-1}m^{-2}$):	250, 400, 550

Only one-half of the matrix treatments have been completed but the data obtained indicate that carbon dioxide additions provide significant growth advantage only at the medium and low irradiance levels. The data also suggest that irradiance levels of 400 $\mu mol\ s^{-1}m^{-2}$ are close to a saturating irradiance level for potatoes and little advantage is obtained with higher irradiance levels. This is particularly important in terms of overall energy efficiency for growing plants in a CELSS. These conclusions are only tentative and must await the completion of all of the matrix combinations.

Our highest tuber production in terms of grams of edible carbohydrate per day has been obtained from the study shown in Figure 3 with plants grown in 38-liter containers for 147 days at 16°C under continuous irradiation. An

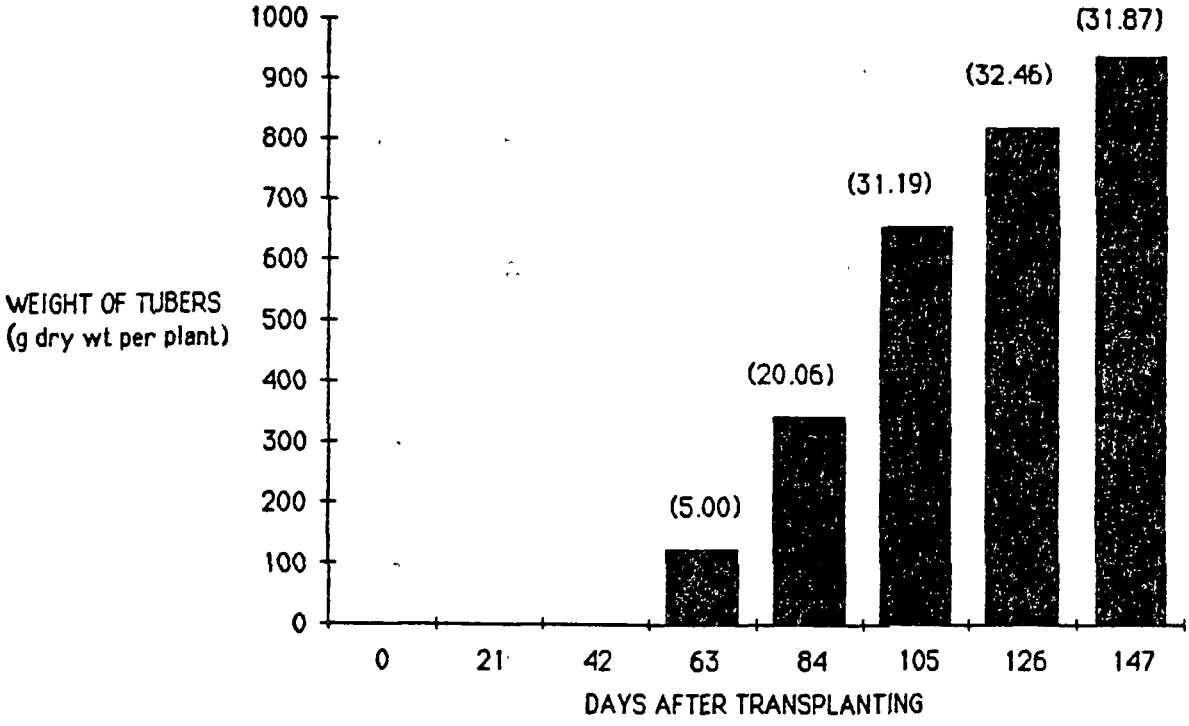

Figure 3. Tuber weight of potatoes at different stages of development. Plants grown with 400 μmol $s^{-1}m^{-2}$ irradiance over 24-hr photoperiod at 16°C and 70% RH. Values in parentheses indicate the g m^{-2} day^{-1} for each harvest.

irradiance level of 400 μmol $s^{-1}m^{-2}$ and relative humidity of 70% were maintained in this study. Each plant was contained by a wire cage having a cross sectional area of 0.2 square meters. These caged plants were separated some in the growing room, thus each plant received some side lighting. The production efficiency at different times during this

study is shown by the values in parentheses above each bar. The production efficiency increased rapidly to 105 days and then was essentially constant between 105 days and the final harvest at 147 days. The production during this period would provide the daily energy requirements for one person from a 23.6 m^2 area. This is calculated with the following assumptions.

One gram dry weight of potatoes = 3.73 k calories

Daily energy requirement of one person = 2800 k calories

The electrical power to provide lighting required for this production was estimated to be 7.2 KW per m^2 of continuous electricity. This is based on the use of high pressure sodium lamps and reflectors as installed at the Phytofarm, a commercial plant growth facility in DeKalb, Illinois. This installation obtains 400 $\mu mol\ s^{-1}m^{-2}$ photosynthetic irradiance with 304 W of high pressure sodium lamps over each m^2. This calculation of irradiance requirement may underestimate the requirement of a production system when plants are grown close together in a closed canopy. However studies suggest that greater irradiation efficiency can be obtained with elevated carbon dioxide levels and by tailoring lighting systems to the particular species being grown in CELSS.

Concurrent studies have been conducted examining the use of soilless culture system for growing potatoes in controlled environments. These have including recirculating solution cultures and continuous misting systems. These systems offer the potential of reducing growing medium requirements and providing more precise control of root-zone nutrient concentrations and pH control. In each case, vigorous shoot growth can be obtained, but induction of tuberization has proved difficult. However, lowering ambient temperatures and reducing solution nitrogen levels has been found to enhance tuber production. Additional studies are in progress directed toward obtaining consistant tuber initiation in liquid systems and comparing tuber production between solid media and liquid culture systems.

In summary it is felt that potatoes do provide a useful plant species for life support systems and should be considered seriously for inclusion in long-term space habitats.

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Optimization of Controlled Environments for Hydroponic Production of leaf Lettuce for Human Life Support in CELSS

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Every scheme of mass recycling within a CELSS features some photosynthetic organism as a figure of central importance in the overall process (Fig. 1). Air revitalization (i.e., CO_2 scavenging and O_2 regeneration) as well as water purification, mineral recycling, and generation of edible biomass all ultimately are driven by the photosynthetic process. Our research project in the food production group of the CELSS program seeks to define optimum conditions for photosynthetic productivity of a higher plant food crop.

Because of our interest in photosynthesis per se, we have elected to work with a salad crop, whose major edible product is new photosynthetic tissue. Under optimum conditions, such a system becomes increasingly productive during the growth cycle. Leaf lettuce is a salad crop for which commercial hydroponic production in controlled environments already is a reality. The cultivars we work with have a harvest index of at least 80% edible biomass, and even though the main photosynthetic product is cellulose, food scientists feel that advances in food

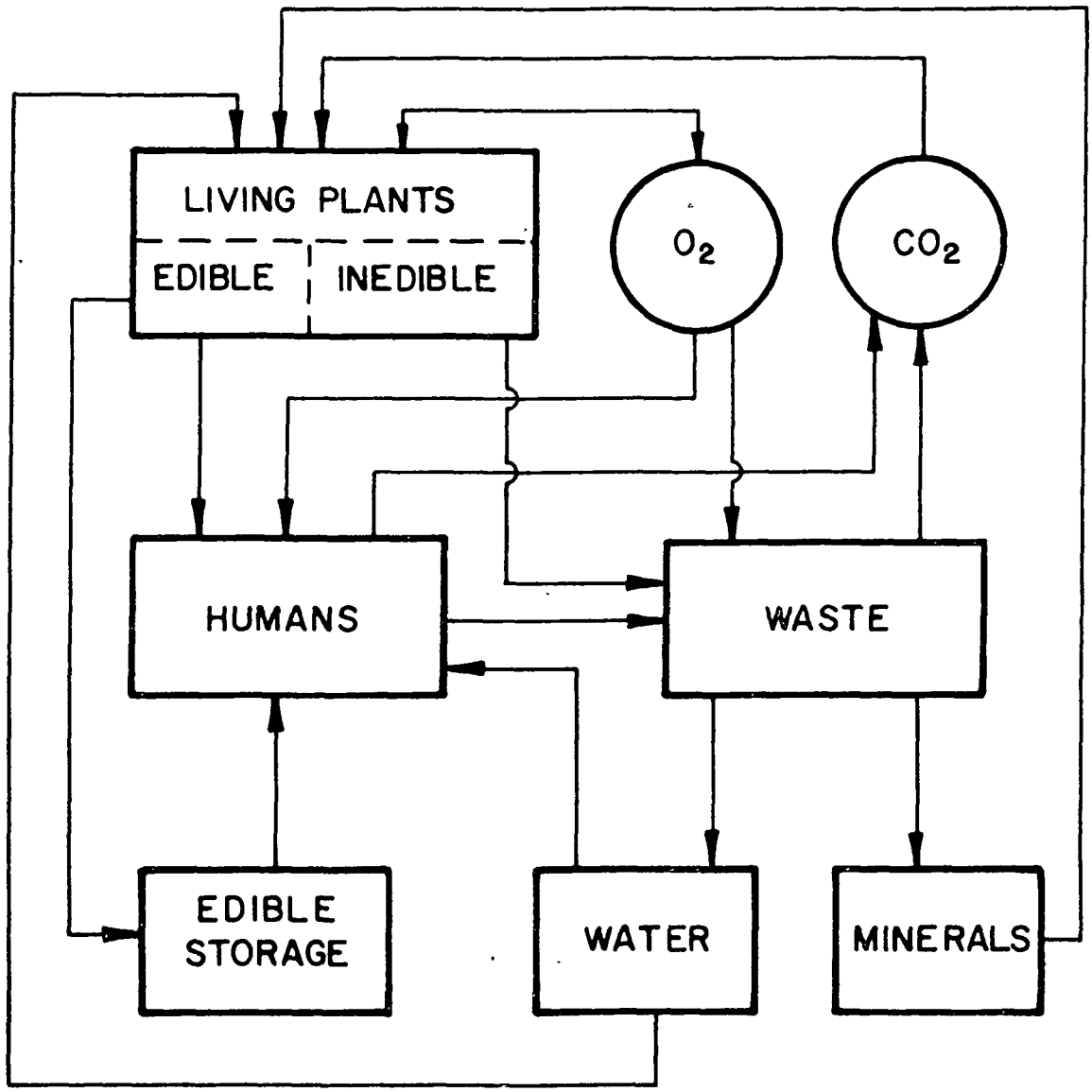

Figure 1. Schematic depiction of a CELSS including photoautotrophic and heterotrophic components, food generation, water purification, air revitalization, and mineral waste recycling.

processing technology will take care of digestibility or palatability limitations of candidate species. Leafy vegetables also provide vitamins and minerals needed for a balanced vegetarian diet that are not provided by protein or calorie crops, and food scientists further tell us that salad crops need not always be consumed fresh, but can be dried, flaked, processed, and incorporated into food bars in appropriate proportions. Leaf lettuce also tends to be quite tolerant of NH_4^+ in nutrient solutions, which may be very useful for recycling nitrogenous human wastes in CELSS. In fact, high nitrogen levels in nutrient solution, including NH_4^+, contribute substantially to the enhanced growth of some lettuce cultivars in response to elevated output from fluorescent (FL) + incandescent (IC) lamps.

Positive growth responses to elevated light and nitrogen prompted us to further test lettuce growth responses to high light from lamp types more energy efficient and longer-lived than fluorescent lamps. A walk-in growth room equipped with water-cooled, high pressure sodium (HPS) vapor, metal halide (MH), and quartz iodide (QI) lamps provided 1100 $\mu mol\ s^{-1}\ m^{-2}$ of photosynthetically-active radiation (PAR), which is roughly half full sunlight level. A recirculating nutrient film system with separate root temperature control also has been installed within the chamber. The use of HPS radiation to grow lettuce is paradoxical: lettuce seems to grow well with HPS as a sole

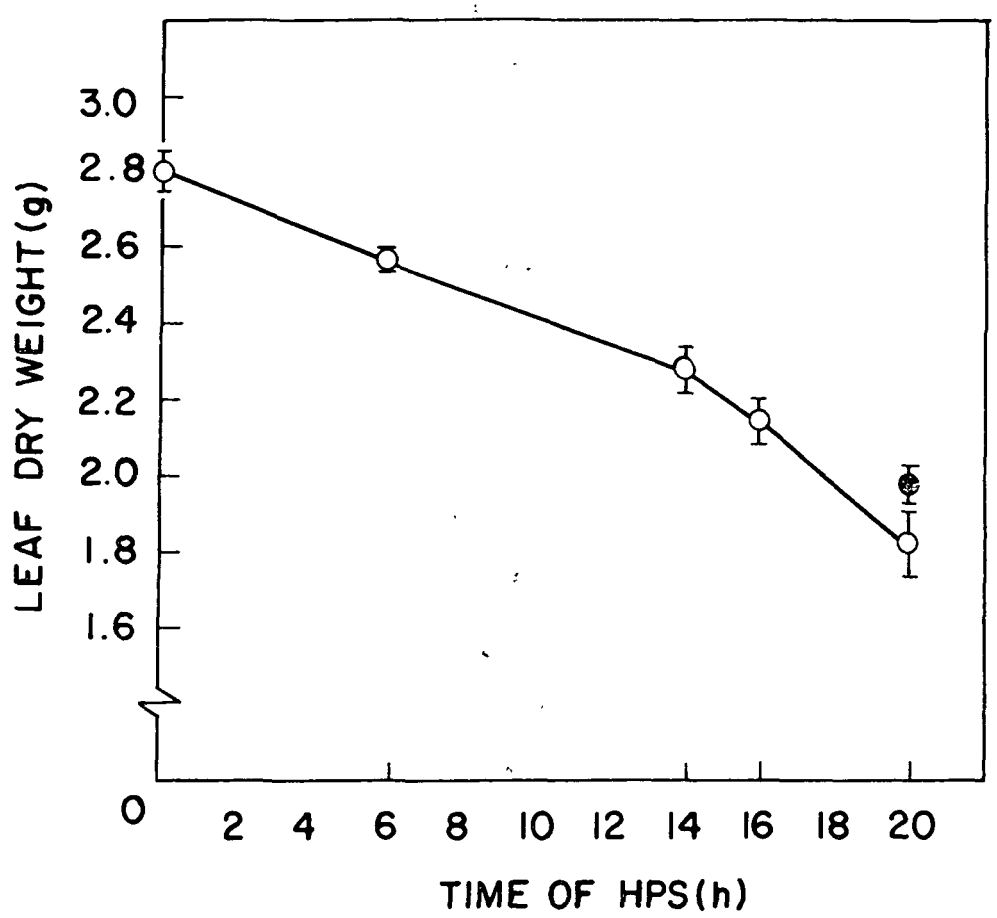

Figure 2. Effect of different times of exposure to high irradiance HPS radiation on leaf dry weight per plant of 'Black-Seeded Simpson' lettuce after 19 days of growth. All treatments indicated by open circles received 20 h day^{-1} of metal halide + quartz iodide radiation at 300 µmol s^{-1} m^{-2} of PAR. When all 3 sources were energized, PPFD was 1100 µmol s^{-1} m^{-2}. The closed symbol represents plant response to 20 h day^{-1} of FL + IC lighting at 750 µmol s^{-1} m^{-2}.

radiation source if irradiance level is kept low, but its response to high irradiance HPS, particularly in combination with other sources, is less clear. Therefore, we are investigating effects of different dosages of HPS in combination with a constant dosage of MH + QI. Nineteen-day-old 'Black-Seeded Simpson' lettuce plants grown for 20 h day^{-1} under HPS + MH + QI become abnormally yellow under this high radiation regime. Doubling the N level and providing N as a mixture of NH_4^+ + NO_3^- instead of just as NO_3^- increased plant size and resulted in slightly less yellowing of inner leaves. If MH + QI were maintained at 20 h day^{-1}, but the HPS reduced to 14 h day^{-1}, the plants become even less yellow, and a little larger than at 20 h HPS. We have extended this reduction in exposure to high irradiance HPS to zero while keeping MH + QI constant, and preliminary evidence suggests that leaf dry weight increases as duration of HPS radiation decreases (Fig. 2). The highest yield obtained has been with MH + QI alone at only 300 µmol s^{-1} m^{-2}. This is substantially greater than the leaf weight of plants grown in a similar culture system under 20 h day^{-1} at 750 µmol s^{-1} m^{-2} of FL + IC lighting. The input wattage of IC in the latter regime was only 8%, whereas that in combination with MH was 31%. That may be a key factor in the greater yield at the lower photosynthetic photon flux density (PPFD) than at the higher PPFD. Nevertheless, none of these are particularly high

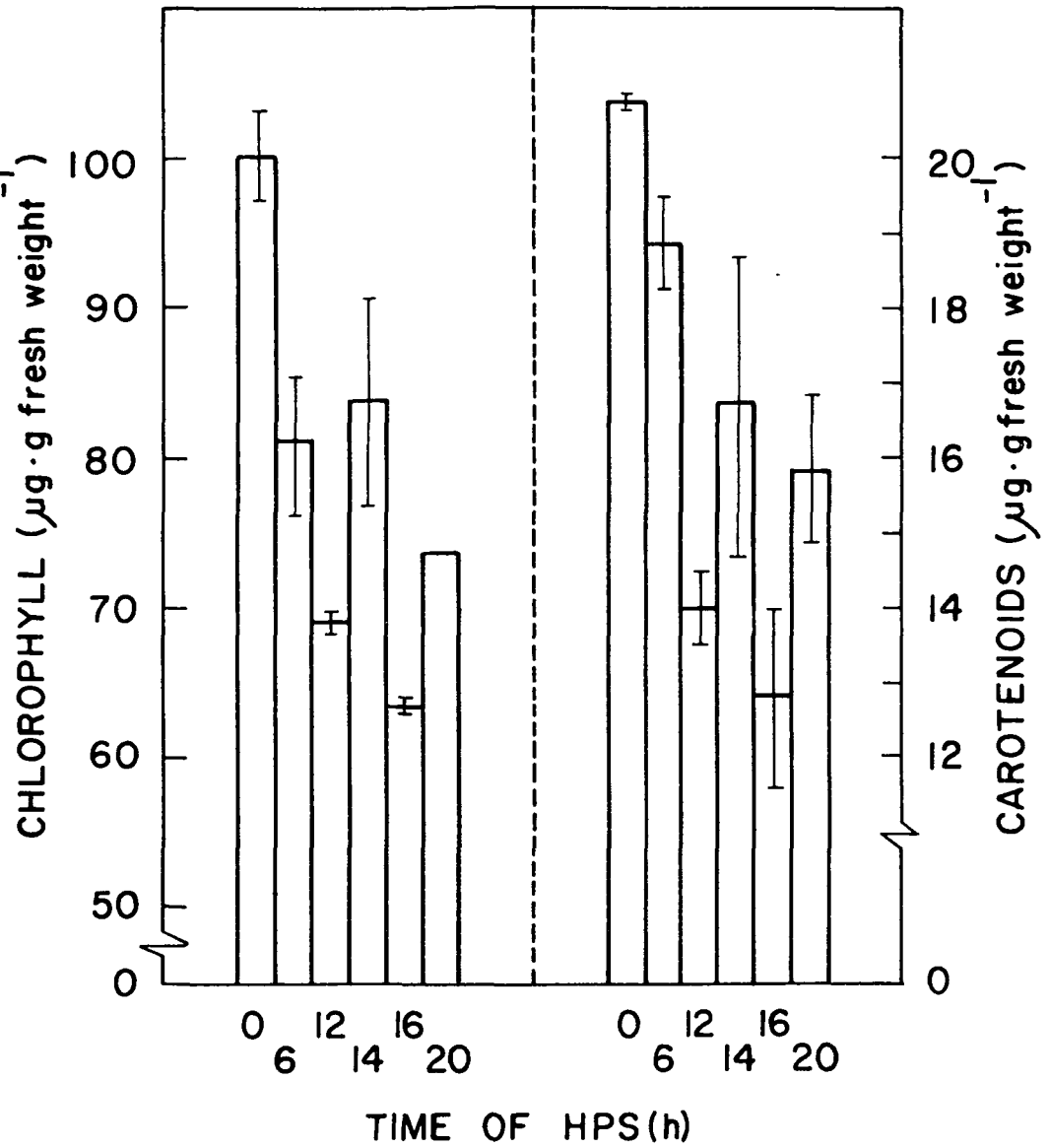

Figure 3. Effect of different times of exposure to high irradiance HPS radiation on specific chlorophyll and specific carotenoid contents of outer leaves from 18-day-old 'Black-Seeded Simpson' lettuce plants.

yields for 19-day-old plants, but they do suggest that high irradiance HPS damages leaf lettuce.

The leaf yellowing caused by excessive HPS radiation may be due either to an increase in yellow pigments or to a decrease in green pigments. Preliminary evidence suggests that specific carotenoid content actually decreases with increasing HPS dosage, as does specific chlorophyll content (Fig. 3), so the HPS effect is a true chlorosis, preventable mainly by avoiding use of HPS lamps, and to a lesser extent by using double-strength N as $NH_4^+ + NO_3^-$. We plan to test effects of low intensity HPS or MH as sole radiation sources on yield and pigment content of leaves. We also are equipping our walk-in chambers with a CO_2 control capability, and feel that defining the proper combinations of high CO_2, long photoperiod, moderate output from the right lamp types, and proper timing of those treatments is needed to consistently obtain superior lettuce yield rates.

We already have demonstrated the feasibility of this approach on a smaller scale, using a second-generation Minitron chamber system developed in our laboratory (Fig. 4). Each cylindrical chamber is 24 inches in diameter, has a 24-inch-high growth height, and transmits radiation from external lamps. A fan in the base of the chamber pulls air down through the center

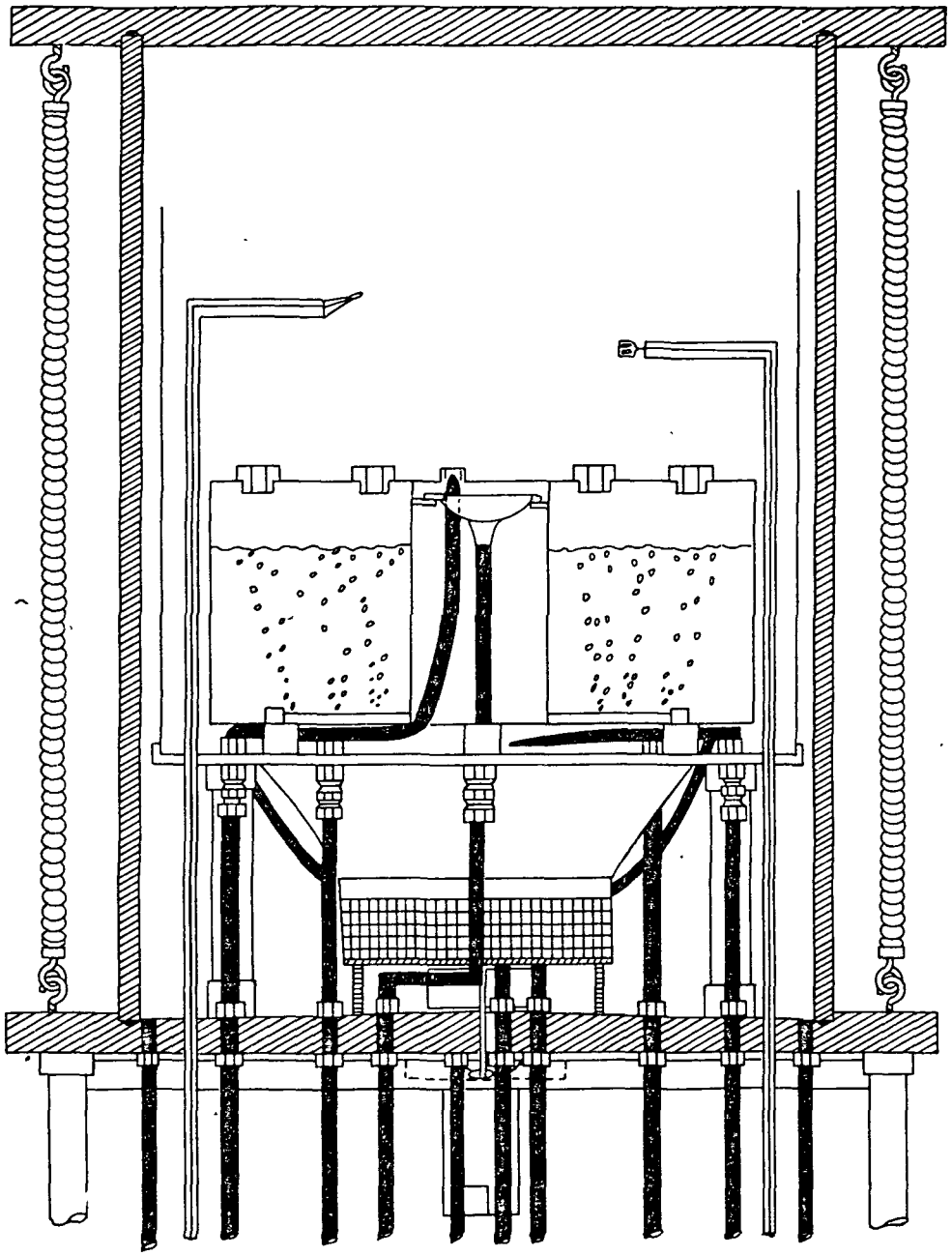

Figure 4. Schematic side view of a Minitron II plant growth chamber for hydroponic growth of plants in a controlled environment, including a flowing, defined atmosphere.

of a donut-shaped hydroponics pot, through the fins of a heat exchanger, outward and upward between the outer wall and a thin, transparent baffle extending above the crop canopy before circulating downward again. Inlet atmosphere is dispersed into the fan stream, and some outlet air is captured in the middle of the donut hole on every downward pass and directed out of the chamber. The lid of the hydroponics pot is o-ring sealed to the container, and each plant holder is o-ring sealed to the lid (Fig. 5), so a fairly air-tight seal is achieved between root and shoot compartments. This is important in providing atmospheres of different composition to roots and shoots, and for measuring gas exchange in each compartment separately. Closed-cell Ethafoam plugs support one seedling in each of the 36 holders available. The depth of nutrient solution in the container is controlled by the length of an overflow tube. Uniform aeration of roots is provided by a circular aquarium wand in the bottom of the container. The root atmosphere is vented separately through an outlet in the lid.

A minimum amount of support equipment is needed for environmental control in the Minitron system (Fig. 6). An oilless, teflon-piston-driven air compressor is used to provide continuous air exchange for root and shoot compartments. The air is first dried to keep H_2O out of the flow valves; it is then Purafil-filtered to eliminate unsaturated hydrocarbons, mixed in

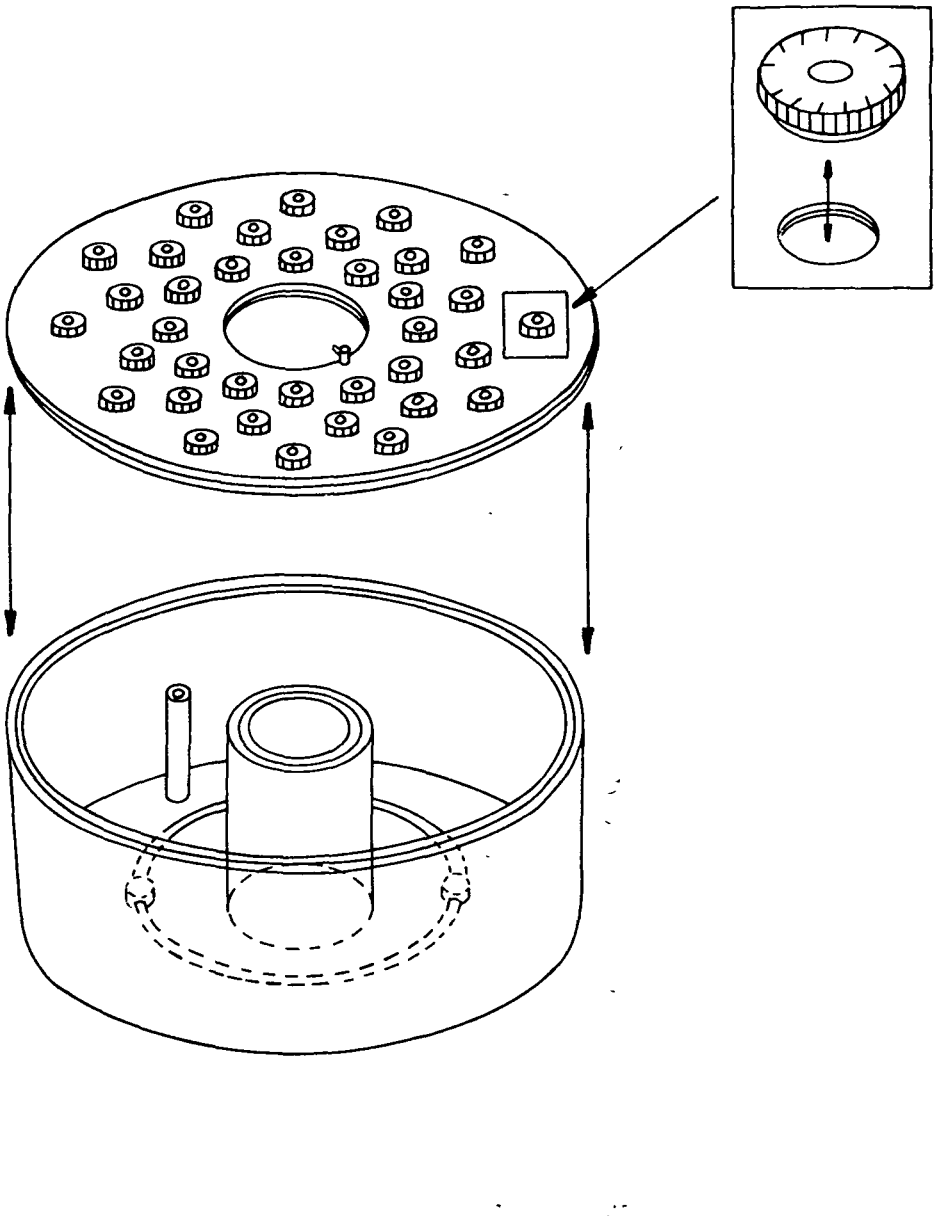

Figure 5. Donut-shaped hydroponics container including o-ring-sealed lid and individual plant holders, overflow tube, and circular aquarium wand.

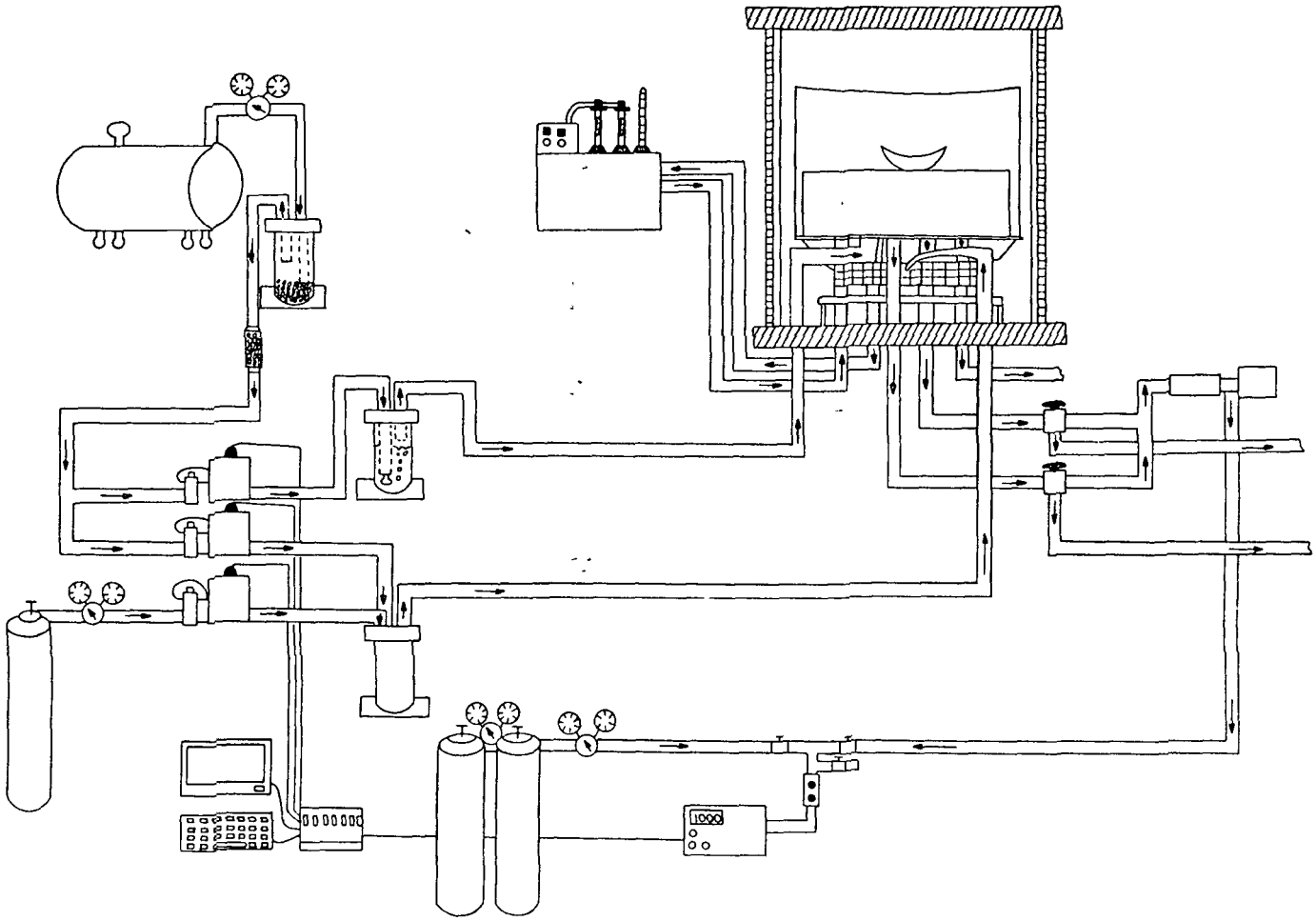

Figure 6. Environmental support equipment for the Minitron II plant growth system, including temperature control system, flowing atmosphere composition and flow rate system, CO_2 infrared gas analyzer, mass flow controller, and dedicated micro-computer.

a pre-determined proportion with pure CO_2, the mixture re-humidified, and metered at a controlled rate into the shoot compartment of the chamber. Air to pass through the root compartment is humidified without additional CO_2 injection. Outlet gases are either vented from the room or directed through an infrared CO_2 gas analyzer (IRGA). A recent acquisition is a computer-assisted mass flow control system, which adjusts a proportioning valve in response to signals from a computer, which in turn takes its cues from the IRGA. Software is being developed to maintain a constant level of CO_2 in the chamber even as plant demand for CO_2 changes. Gas exchange rates will be determined by the amount of CO_2 required to maintain CO_2 homeostasis in the chamber. We have used elevated CO_2 in the chambers for some time, but not with this sophisticated degree of CO_2 control. A thermostatted water bath circulates coolant through the chamber heat exchanger, and is equipped with a photocell and two thermoregulators so that it can automatically switch from day to night set-point temperatures and vice versa.

The inside of the chambers usually is covered with condensation during the dark period, but this soon burns off under a radiation load. Output from supplemental lamps is filtered through several inches of water to alleviate the load on the heat exchange system. Because growth space within the chambers is limited, we do not carry all 36 of the original

seedlings to harvest. Instead, a fixed number are harvested at regular intervals so that those remaining are uniformly spaced. This makes growth dynamics analysis possible, which is a powerful tool for determining productivity rates, photosynthetic efficiency, and how they change over time.

In fact, our experimental approach involves measuring growth dynamics at different stages of the growth curve. Lettuce growth follows a sigmoid pattern of cumulative growth. Lag and plateau phases are not particularly active periods of biomass assimilation. In our studies, we generally harvest while the plants are still in exponential growth, but the lag represents a significant delay. It will be helpful to find ways to shorten it, but to date most of our effort has been directed toward maximizing exponential growth.

After a lag phase of about 11 days, 'Waldmann's Green' leaf lettuce enters a shallow rate of exponential growth at 450 $\mu mol\ s^{-1}\ m^{-2}$ of PAR and 350 $\mu l\ l^{-1}$ CO_2, and this response is quite uniform in 2 separate Minitron chambers (Fig. 7). However, growth was increased by raising CO_2 in one chamber to 1000 $\mu l\ l^{-1}$. In another experiment conducted at 350 $\mu l\ l^{-1}$ CO_2, one chamber was exposed to a PPFD of 925 and another to 450 $\mu mol\ s^{-1}\ m^{-2}$ for 20 h day^{-1}, and the lettuce under higher light yielded better (Fig. 8). This semilog plot includes only the

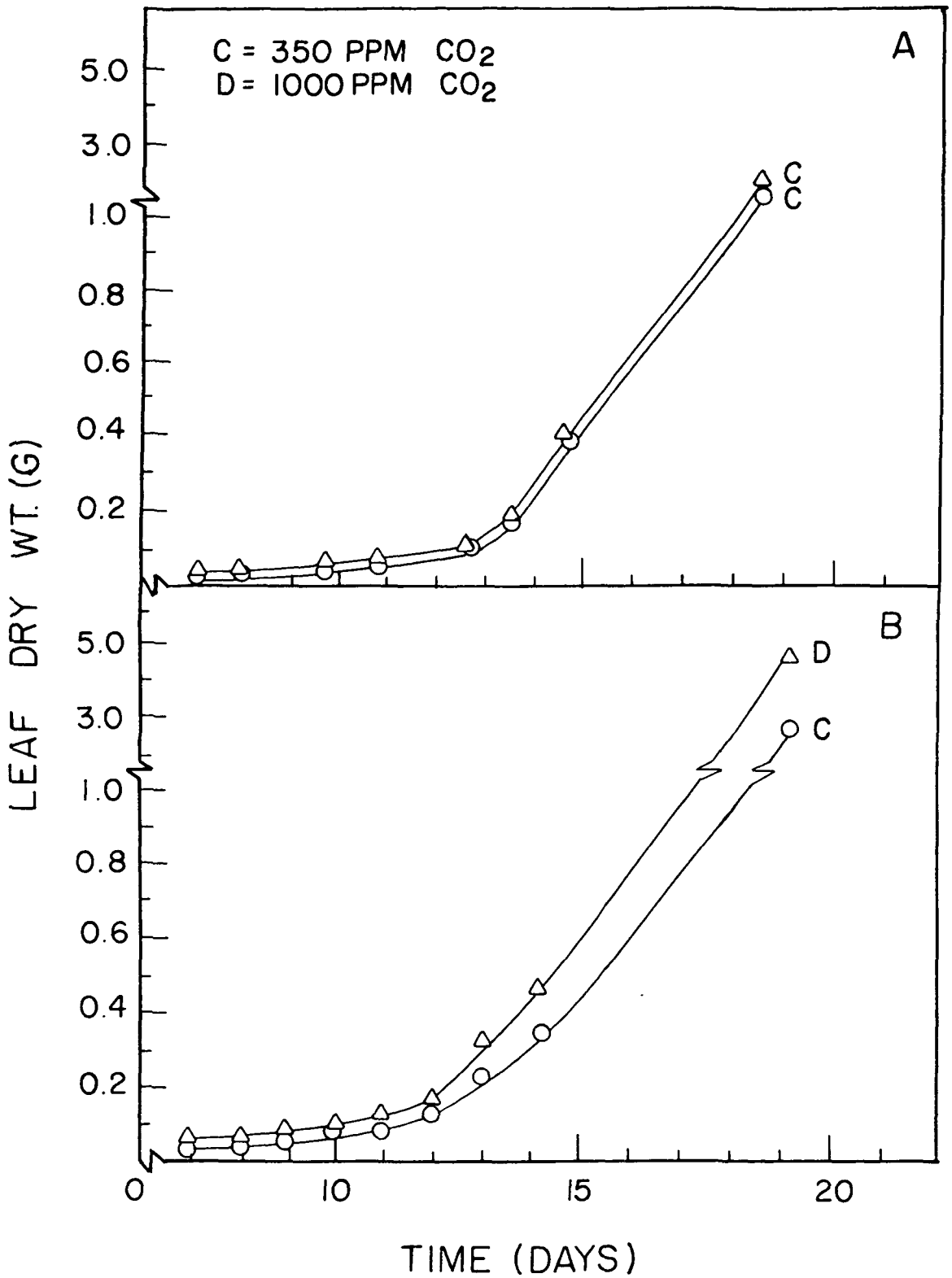

Figure 7. Growth profile of 'Waldmann's Green' leaf lettuce at 450 µmol s^{-1} m^{-2} of PAR + 350 µl l^{-1} CO_2 in 2 separate Minitron II chambers (top), and at 1000 µl l^{-1} CO_2 in one chamber and at 350 in another, both at the same PPFD (bottom).

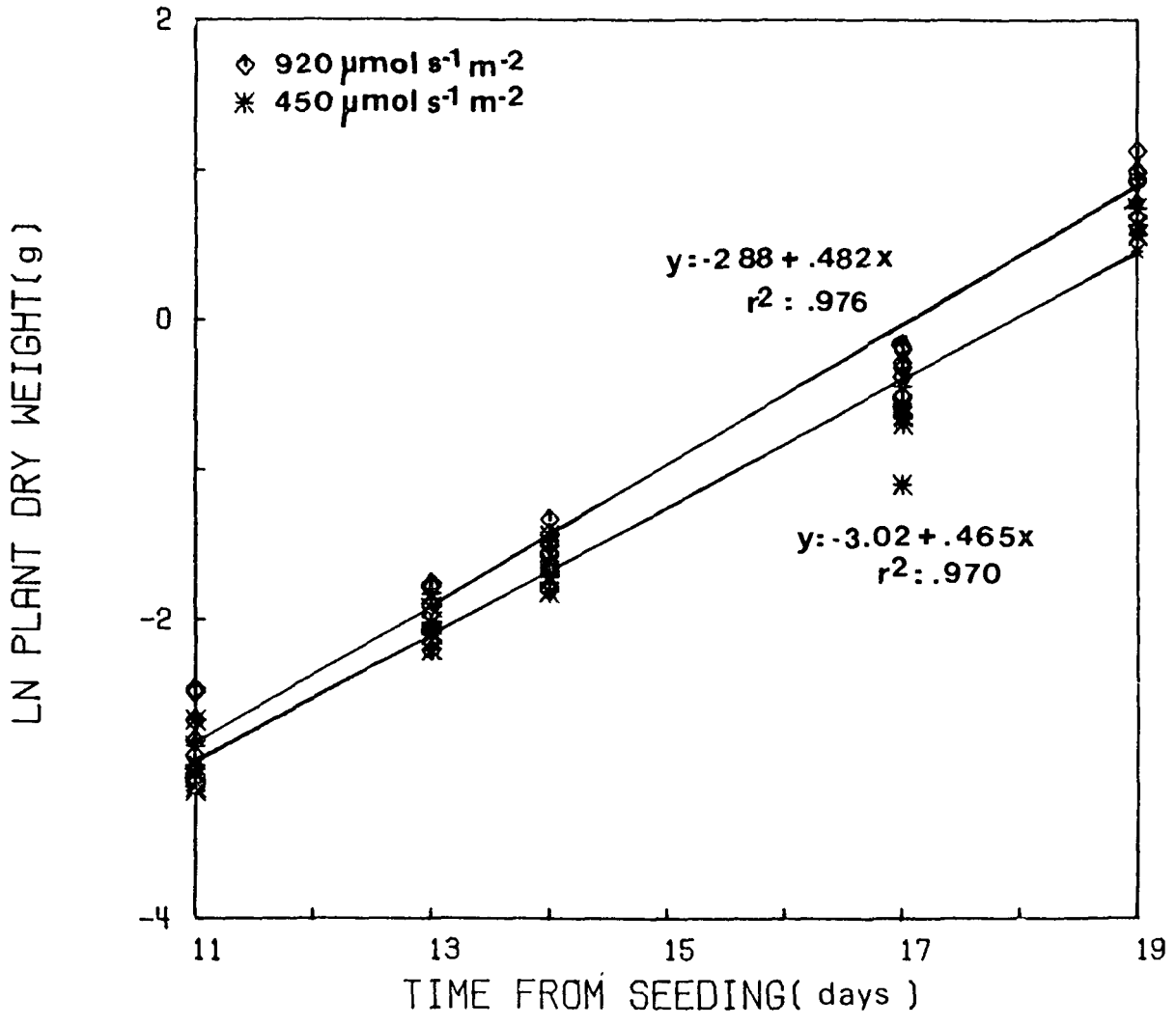

Figure 8. Semilog plot of plant dry weight over time for 'Waldmann's Green' leaf lettuce during exponential growth under either 920 or 450 µmol s^{-1} m^{-2} at 350 µl l^{-1} CO_2.

exponential phase of growth. This type of plot straightens out curved lines, and its slope represents the relative growth rate (RGR) of the plants. In fact, the coefficient of the X term in the regression equation is mean RGR over that time interval. A comparison of RGR at high CO_2 + low light vs. that at low CO_2 + high light indicates that the cheaper CO_2 is slightly more effective than the high light when each are used alone.

When light and CO_2 were enhanced simultaneously, such as CO_2 to 1000 $\mu l\ l^{-1}$ and PPFD to 905 $\mu mol\ s^{-1}\ m^{-2}$, there was a synergistic interaction leading to enhanced RGR and yield (Fig. 9). When CO_2 was raised to 1500 $\mu l\ l^{-1}$, exponential growth was enhanced even further. To determine whether energy and resources can be saved, these optimizing treatments were initiated either 3 days before exponential growth normally begins, or 2 days after. Starting treatment early clearly had no benefit, and starting it late caused the exponential rise to lag. For treatment initiated on day 11, RGR during early exponential growth is extremely high (Table 1); in fact, for high light + high CO_2, the period from about 11 to 13 days gave the highest RGRs we have measured! Unfortunately, they decline rapidly, and one wonders whether there is any need to continue high light treatment much beyond day 13. We are investigating this possibility.

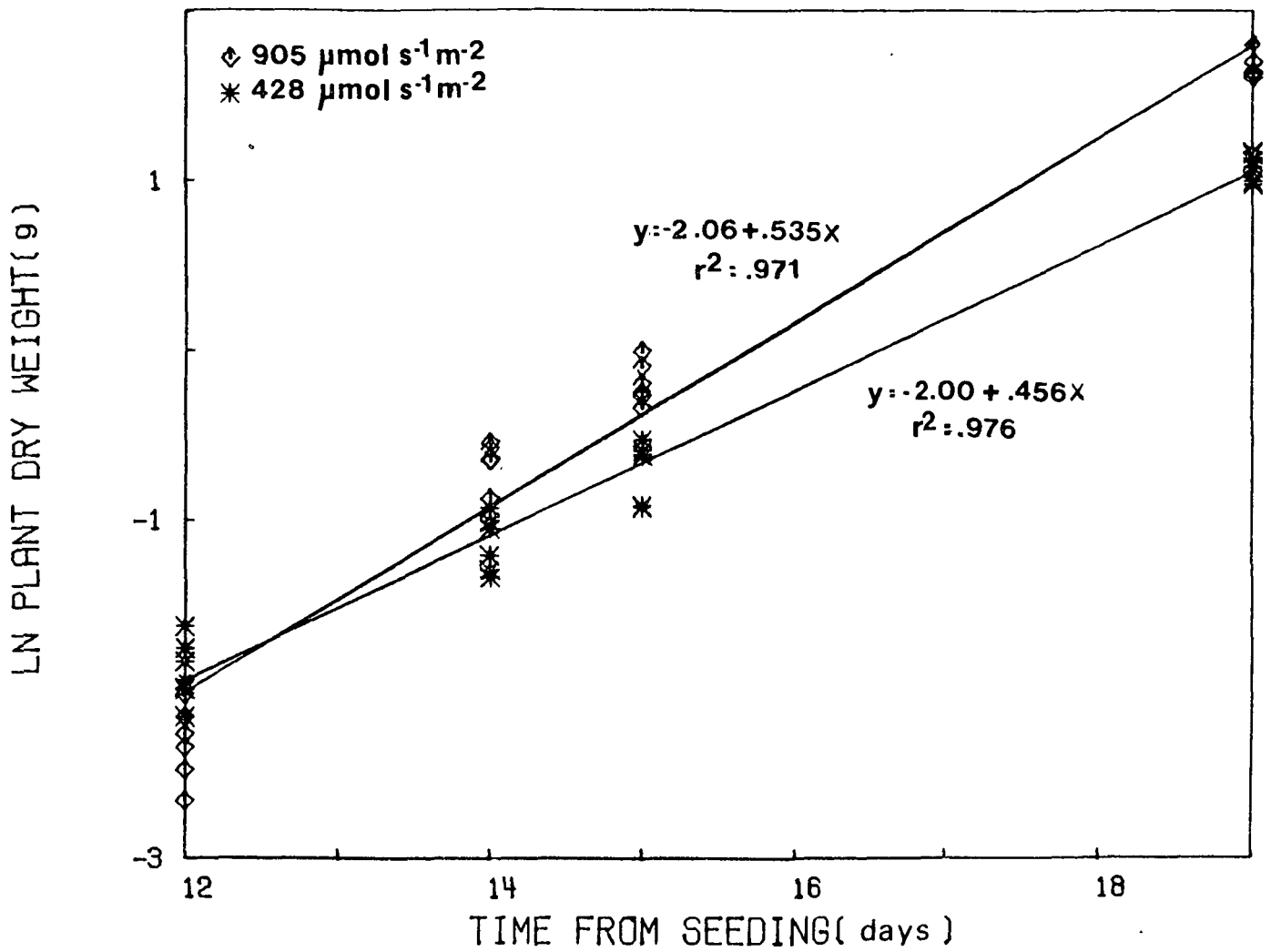

Figure 9. Effect of simultaneous enhancement of CO_2 (to 1000 µl l^{-1}) and PPFD (to 905 µmol s^{-1} m^{-2}) on exponential growth rate of 'Waldmann's Green' leaf lettuce.

Table 1. Change in RGR of 'Waldmann's Green' during exponential growth as a function of PPFD at high CO_2.

Growth period (days)	Relative growth rate @ 1500 $\mu l \cdot l^{-1}$ CO_2	
	Photosynthetic photon flux density ($\mu mol \cdot s^{-1} \cdot m^{-2}$)	
	450	900
	($mg \cdot g^{-1} \cdot day^{-1}$)	
12-13	603	909
13-15	544	562
15-19	416	501

We also are interested in the spectral emission of lamps used with the Minitron system. High dosages of HPS radiation did not work well here either, but we have had some success with various combinations of IC, MH, and FL lamps. For instance, when low-PPFD MH was compared with equivalent PPFD from IC + FL, lettuce yielded no better under one regime than the other. However, if PPFD was increased to 800 $\mu mol\ s^{-1}\ m^{-2}$ for both regimes in the presence of high CO_2, the IC/FL source was superior to the MH source (Fig. 10). Whether leaf lettuce actually prefers wavelengths present in the emissions from incandescent lamps currently is under investigation. Although incandescent lamp efficiency is low, improvements are forthcoming. We now use "Capsylite" IC lamps, which, unlike standard flood lamps, maintain most of their output as they age.

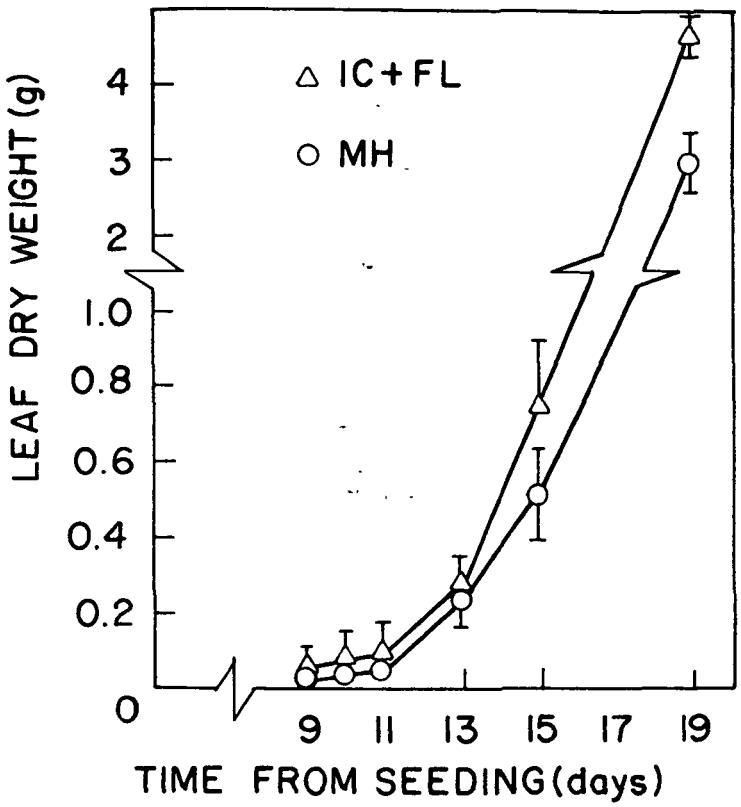

Figure 10. Growth profile of 'Waldmann's Green' lettuce under IC (68% input wattage) + FL radiation (triangles) vs. MH radiation (circles). PPFD of both lighting regimes was 800 μmol s^{-1} m^{-2}.

A lettuce crop grown for 19 days in a Minitron has not quite closed its leaf canopy at harvest. However, lettuce grown for 23 days develops a tightly solid foliar canopy. This mutual crowding greatly diminishes the RGR of individual plants during their last few days of growth, even under optimizing conditions, but those plants still exhibit superior growth compared to widely-spaced plants grown under standard growth chamber conditions (Table 2).

Table 2. Effect of elevated CO_2 concentrations and 900 $\mu mol\ s^{-1}\ m^{-2}$ of PAR on various growth parameters of 23-day-old 'Waldmann's Green' leaf lettuce.

Growth parameter	CO_2 concentration ($\mu l \cdot l^{-1}$)	
	1000	1500
	($g \cdot plant^{-1}$)	
Leaf fresh weight	96.00 ± 5.22 a[z]	106.48 ± 8.60 b
Leaf dry weight	8.98 ± 0.33 a	10.47 ± 0.41 b
Stem dry weight	0.59 ± 0.06 a	0.79 ± 0.09 b
Root dry weight	1.47 ± 0.07 a	1.75 ± 0.04 b
Plant dry weight	11.05 ± 0.17 a	13.01 ± 0.21 b

[z]Mean separation within rows by t-test at the 5% level of significance.

We have begun to test effects of plant growth regulators (PGRs) on lettuce growth concomitant with optimizing environmental conditions. For example, the 30-carbon primary

alcohol triacontanol tends to have promotive effects on yield, but formulation problems still prevent consistent performance with this chemical. With fresh, colloidally-dispersed triacontanol sprayed on during lag phase, stimulatory effects disappear after the first few days of exponential growth. With or without triacontanol, per plant yield of 'Waldmann's Green' under optimizing conditions is excellent after 19 days (Table 3). When expressed on an area-occupied basis, crop growth rate (CGR) during exponential growth was 55 to 60 gDW m^{-2} day^{-1}, and this does not even involve complete closure of the canopy yet. This compares with a typical CGR of 2.6 g m^{-2} day^{-1} over an entire production cycle for lettuce under field conditions.

In conclusion, we plan to extend our investigations of optimum lamp types; evaluate effects of other PGRs; fine-tune the timing of application of optimizing treatments with the aim of conserving energy and resources; use gas exchange rates of small crop canopies in the Minitrons to identify short and long-term plant response to optimizing treatments; and attempt to shorten the lag phase of the growth curve. Learning how to effectively modulate photosynthetic activity of vegetative canopies on a somewhat larger scale by manipulating light level and duration and/or CO_2 level could have important implications for systems control in CELSS.

Table 3. Effects of triacontanol applied twice during lag phase on various growth parameters of 19-day-old 'Waldmann's Green' lettuce. Plants also were exposed to high CO_2/high light treatment beginning on day 11.

Growth parameter	-TRIA	$+10^{-7}$ g l^{-1} TRIA
	($g \cdot plant^{-1}$)	
Leaf fresh weight	49.30 ± 6.76 a[z]A[y]	57.64 ± 3.77 bB
Leaf dry weight	4.93 ± 0.64 aA	5.62 ± 0.52 aB
Stem dry weight	0.34 ± 0.08 aA	0.36 ± 0.05 aA
Root dry weight	0.97 ± 0.08 aA	1.18 ± 0.08 bB
Plant dry weight	6.25 ± 0.72 aA	6.99 ± 0.65 aB
	($g \cdot m^{-2} \cdot day^{-1}$)	
Crop growth rate	55.05 ± 7.04 aA	60.48 ± 9.07 aA

[z]Different lower case letters within rows different according to t-test at the 5% level of significance.

[y]Different upper case letters within rows different according to t-test at the 10% level of significance.

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CLOSED CULTURE PLANT STUDIES: IMPLICATIONS FOR CELSS

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Abstract:

Arabidopsis plants were grown in closed cultures similar to those used in space experiments. A shift in metabolism from photosynthesis to respiration is indicated by the accumulation of CO_2 in the culture atmosphere. Reproductive growth is suppressed. Plant growth and development is apparently related to the atmospheric volume available to each plant. The implications of these findings to CELSS were as follows: 1) need for an open culture having ample gas exchange, 2) CO_2 levels be maintained within prescribed limits, 3) the minimum atmospheric volume required for each plant is thus dependent on the precision of the gas monitors and of the subsystems used used to maintain appropriate levels of various atmospheric components, and 4) volatiles such as ethylene and terpenes emanating from plants be monitored and reduced to benign concentrations.

Introduction:

In a controlled ecological life support system (CELSS), the fundamental system premise is based on the classic photosynthesis reactions of green plants. Plants exposed to light of proper wave lengths and intensities convert CO_2 and water into fixed

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carbon compounds with a simultaneous release of O_2 into the atmosphere. As a result of this photosynthetic reaction, the fixed carbon compounds form the food to be eaten and the uptake of CO_2 and production of O_2 replenishes the air for astronauts. The equation is presented below in an incomplete and simplified form:

$$6\ CO_2 + 6\ H_2O \dashrightarrow C_6H_{12}O_6 + 6\ O_2$$

This reaction occurs under planet earth conditions where the CO_2 atmospheric concentration is around 300 ppm and O_2 around 20 per cent.

As an example, let us take a green living plant and place it into a small gas-tight glass container. This container when sealed would have the green living plant, a supply of air, water and nutrients for the plant. Being a closed system, no materials are taken out or added. However, energy is supplied in the form of light. Under these conditions, one would expect - following the above photosynthesis equation - the plant to photosynthesize taking up the CO_2 present in the atmosphere until CO_2 is reduced to a low concentration. In theory, an analysis of the gases at this point would be predicted to have a slight increase in O_2 concentration with a concomittent decrease in the CO_2 level. In practice, this is not the case. In closed systems, such as that described, CO_2 was found to increase (Hoshizaki, 1984, Cowles et al., 1984) and O_2 decrease (Cowles et al., 1984). These results

are completely and diametrically opposite from those predicted. Thus, for CELSS being discussed at this meeting, I believe these results have high relevance.

This paper will present background information and various topic matters that relate to this closed system phenomenon. In these discussions, I will attempt to relate and highlight the various bits of information as it might apply to CELSS.

Previous Research and Results:

Tightly sealed test tubes (Hoshizaki, 1982, 1984) and small volume growth chambers (Merkys et al., 1981) have been used to test the effect of space environment, real or simulated, on many species of plants. When attempts were made to carry these plants through a life cycle, there was a general failure to do so. This was especially true in the Soviet space efforts with the plant Arabidopsis thaliana, (L.) Heynh (Merkys et al., 1981). It was only until a "ventilated" open growth chamber was used, that Merkys et al., 1984, were able to successfully grow arabidopsis from seed to seed.

Our ground based experiments with the same species had indicated earlier that a culture system having ample gas exchange with the ambient was required for growing arabidopsis plants from seed to seed in vitro (Hoshizaki, 1982). Whenever the in vitro

cultures were truly sealed and closed, having no gas exchange with ambient, the arabidopsis plants growing inside always failed to grow normally and never set seeds. Thus, we found 1) normal vegetative growth occurred only in cultures having gas exchange with the surrounding ambient atmosphere, 2) abnormal growth occurred in closed gas-tight cultures, 3) reproduction and completion of the life cycle (production of viable seeds) only occurred in cultures having gas exchange with ambient and 4) growth rate, stages of life cycle attained and final size of plants in closed cultures related to the atmospheric volumes surrounding each plant.

The implication of these results is the need for small cultures to have ample gas exchange with the ambient atmosphere surrounding the culture system.

We initially speculated that the probable cause of the plant responses was most likely related to the initial amount of CO_2 available to the plants in the closed in vitro culture. In a follow-up experiment we measured weekly, over an 8-week period, the level of atmospheric CO_2 in closed in vitro cultures (Hoshizaki, 1984). Arabidopsis seeds were planted one to each container and the growth and CO_2 levels were recorded. As expected, CO_2 levels decreased at the end of the first week with the lowest recorded value of 147 ppm. Surprizingly, the CO_2

level increased from this time on and reached a level of 0. 5 percent for the weekly measured cultures and 15 percent for those cultures sampled only at the start and end of the 8-week experiment (Figure 1). The plants continued to grow producing leaves and elongating their stems during the period when the CO_2 levels were around 0.5 percent or 5000 ppm (cf Fig. 1 and Fig. 2) Why did the CO_2 level rise to such high levels? Did photosynthesis stop when CO_2 levels rose above a critical level? Did the plants enter a heterotrophic phase as previously reported by Brown et al, 1979? Ample light and appropriate temperatures were being given to these plants. Do these results mean that CO_2 levels must be maintained within prescribed limits if plants are to grow normally?

At the end of the experiment, lower values of CO_2 were measured but these were considered to be the result of gas leaks induced by the multiple sampling through the rubber septum. Bacteriological tests made for possible culture contamination as a source of the CO_2 proved negative.

A similar increase of CO_2 levels was found in the space shuttle (STS-3) experiments where pine seedlings and germinating oats and beans were flown (Cowles et al., 1982). The pine plants placed in the plant growth container (PGC) as 4-day old seedlings were thus past the initial germination stage where high respiration occurs.

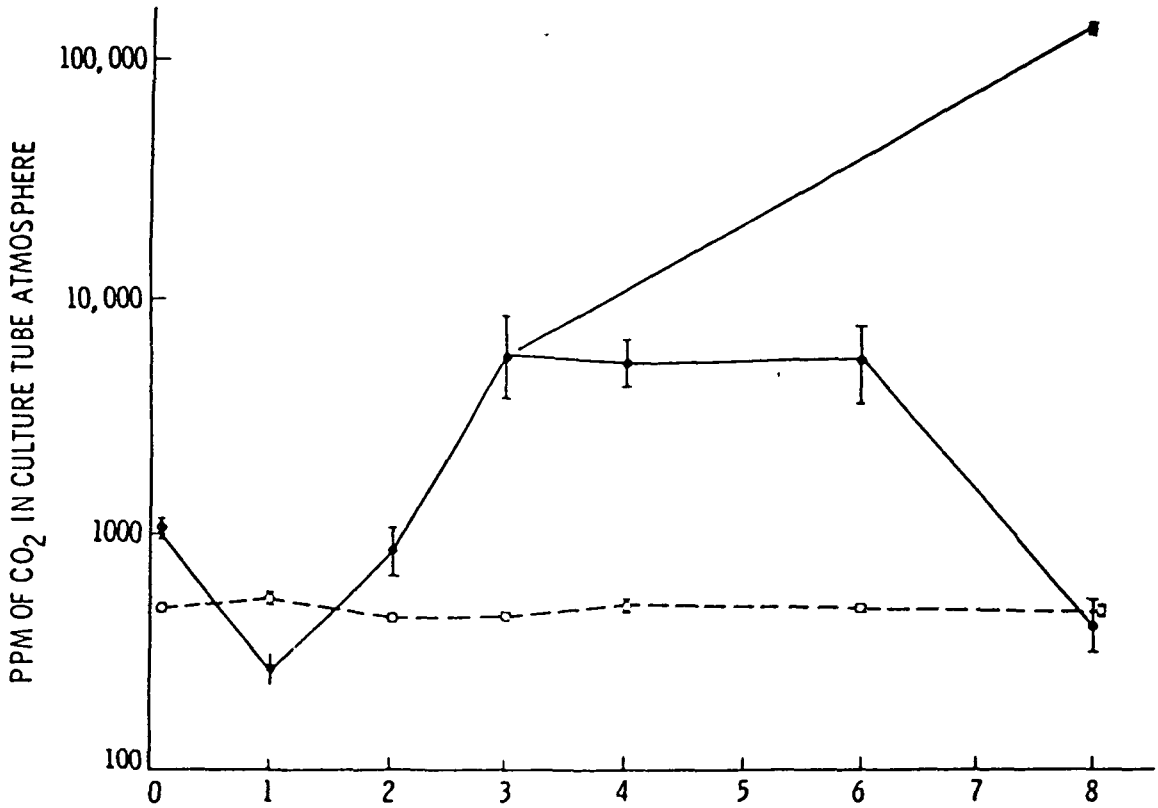

Figure 1. Concentration of carbon dioxide over an 8-week period in the atmosphere of *in vitro* cultures containing *Arabidopsis thaliana* (L.) Henyh. plants. Seeds were planted at 0 week. Closed cultures (•) were sealed with vinylidene polymer film held in place by rubber bands. Open cultures (o) were plugged with polyurethane foam permitting air exchange with ambient. Each value represents the mean ± standard error of CO_2 concentration detected in 40 µl samples drawn from the head space of cultures. Open culture values for 0 week and 8 weeks are from 4 cultures. Closed culture values for 0 week are also from 4 cultures. All others are from 2 cultures. The solid line drawn from week 3 to week 8 represents the assumed curve for cultures sampled only at the beginning and at the end of the experiment.

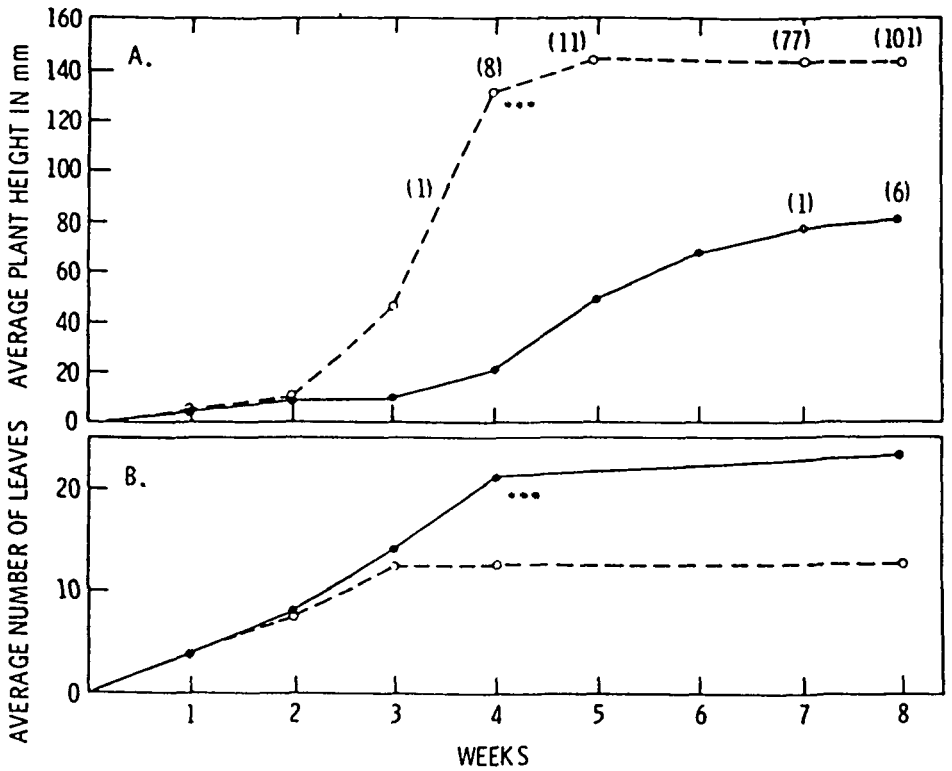

Figure 2. Growth and reproduction response of Arabidopsis thaliana (L.) Henyh planted as a seed and grown over an 8-week period in open (o) or closed (•) in vitro cultures. A. Plant height in mm and total number of fertile seed pods ( ) counted on plants of each treatment. Depth of agar medium in the culture tubes was around 50 mm thus leaving 150 mm for stem growth. B. Number of leaves per plant. Leaves with length shorter than 2 mm were excluded from the count. Each value represents the mean derived from 4 cultures. ***Difference between closed and open cultures at P <0.001.

These plants were green and under light intensities provided would be capable of photosynthesizing. The oats and bean plants were green and had leaves by the end of the 8-day flight experiment. These plants also appeared to be capable of photosynthesis. However, CO_2 levels from 1.41 to 4.92 percent were measured in flight containers and 2.18 to 4.07 percent in ground controls at the end of 8 days. The CO_2 source was attributed to respiration. The O_2 levels which were initially 24.1 to 24.5 percent in the flight containers decreased to 9.7 to 16.3 percent. Similar differences were found in the ground controls. If one assumes a 1:1 stoichiometry of O_2 uptake to CO_2 production in respiration , a discrepancy appears in the amount of CO_2 found. At this time, one can only to speculate about the missing CO_2.

In the arabidopsis closed in vitro cultures, a 15 percent CO_2 level was measured. It was speculated (Hoshizaki, 1984) that an ethylene-CO_2 feedback occurred resulting in a high production of CO_2 by the plants. In a closed system such as CELSS, the same volume of air would be retained for the duration of the mission. Unless steps were taken to remove such compounds, it is more than likely that a similar response might occur. In the STS-3 experiment, ethylene was detected in one of the PGC (Cowles et al., 1984).

Thus, there appears to be a need to carefully monitor the levels of volatiles such as ethylene and terpenes and develop systems to scrub these types of compounds from the atmosphere.

Volume Relationship Relative to Plant Material:

The results of air volume/closed culture experiments performed with arabidopsis indicate that a minimum volume of atmosphere to plant biomass may exist (Hoshizaki, 1984). Below this volume, abnormal growth and metabolic responses may occur. Two closed systems, the STS-3 PGC and the sealed test tubes will now be compared. Mung bean seeds were planted in the PGC (Cowles et al., 1984) and arabidopsis seeds in the sealed test tubes (Hoshizaki, 1984). Seed volumes are estimated from measurements taken of dried seeds. These are only approximate and are presented for the purpose of demonstrating broad generalities. Precise volumes are not implied. Table 1 compares the two closed plant growth systems. The PGC with mung beans is far and above over-loaded in terms of biomass as compared to the sealed tube with arabidopsis. This is expected since the PGC was designed for short duration missions, whereas the arabidopsis culture was selected for long term seed production experiments. The arabidopsis system used here for comparison did permit the arabidopsis plant to complete its life cycle. However, the seed production was low. The estimated value of 550 liters required as air volume to grow mung beans to seed in a closed system is presented to give the reader a grasp of the volume of air

TABLE 1.

COMPARISON OF 2 CLOSED PLANT GROWTH SYSTEMS

	(1) Plant Growth Container	(2) Sealed Test Tube
Plant	Mung bean	Arabidopsis
Atmospheric volume of container - cm^3	1224	455
No. of plants in container	16	1
Atmospheric volume per plant - cm^3	76.5	455
(3) Dry seed volume - cm^3	7.2×10^{-2}	6.0×10^{-5}
(4) Atmospheric volume required by one plant for seed production - cm^3	550×10^3	455

(1) Cowles et al., 1984, (2) Hoshizaki, 1984, (3) Volume estimated from measuring the length, width and thickness of a dry seed, (4) Volume of air required for mung seed production in a closed system as estimated from values obtained from arabidopsis data.

required. The use of the very small seeded arabidopsis plant tends to mislead investigators in judging media and gas volume requirements.

Volume Requirements Relative to CO_2 and the CELSS Plant Growth Chamber:

Two closed systems of vastly different sizes will be used to discuss volume effects of CO_2. The planet earth and a 4-person CELSS unit will be compared. The 97 percent food closure unit of CELSS is selected for our example, and such a system will have only 3 percent of the food provided by drawing from stores or obtained by re-supply (Gustan and Vinopal, 1982).

The rest of the food, 97 percent, will be produced in the CELSS unit by recycling materials within the space habitation unit. The comparison is made between planet earth and the defined CELSS system to again highlight the magnitude of change imposed on plants growing in a closed system; and conversely to highlight the biological demands placed on such a CELSS plant growth chamber.

The values entered in Table 2 were derived from various sources and are expressed in a manner as to simplify comparisons. For the planet earth, the CO_2 turnover period is estimated to be between 5 and 10 years. Only the carbon exchange between the

TABLE 2

EFFECT OF PLANT CO_2 UPTAKE ON EARTH AND CELSS ATMOSPHERES

	Plant earth	4 person CELSS
Atmospheric volume - M^3	4.3×10^{18} (1)	1.5×10^2 (2)
CO_2 turnover time	5-10* years (3)	45 min (4)
Supply left after one min	5-10 yr supply no detectable change	44 min supply ~2% decrease

(1) Estimation based on air height being 7600 m when all air is at one atmosphere pressure, (2) Gustan and Vinopal, 1982, (3) Emanuel et al., 1984, (4) Tibbitts and Krizek, 1978. *Only carbon exchange between earth atmosphere and terrestrial plants are considered for simplification and the most conservative time estimates were used. Inclusion of carbonates in the ocean and other carbon sources will greatly increase turnover time.

earth's atmosphere and the terrestrial plants were considered to simplify the discussion and the most conservative time estimates were used. The values used were obtained and then modified from Emanuel et al., 1984. Inclusion of the carbonates in the ocean and other sources will greatly increase the turnover time. On the other hand, the CO_2 turnover time in CELSS is projected to be 45 min using values calculated from the pulldown of CO_2 in a reach-in growth chamber from 320 to 306 ppm in 2 min by butterhead lettuce (Tibbitts and Krizek, 1978). In one min there will be a 6-7 ppm decrease in the CO_2 supply which must then be detected and replenished by the CELSS gas detection and concentration maintenance unit. A ± 20 ppm variance can be tolerated by plant but this will more than likely reduced harvest yield. For the atmosphere of earth, there will be no detectable global change after one min since the CO_2 turnover time is estimated at 5-10 years. A large buffer system for CO_2 thus exists for the plants of earth. In a CELSS, such a buffer is non-existent. Furthermore, the greater the plant biomass is to the CELSS atmospheric volume, the greater will be the replacement rate. These are engineering problems, but it does indicate the possibility of rapid changes in CO_2 levels in a relatively short time frame. From this, the minimum atmospheric volume required for each plant in a CELSS will depend in part on the precision of the CO_2 gas monitor and the subsystems replenishing the CO_2 gas.

PROTOTYPE PLANT GROWTH CHAMBER OF CELSS QUALITY:

Plant growth chambers in use today are generally very leaky. For a reach-in chamber, a leakage rate equal to 30 percent of the internal volume in 5 min have been measured (Tibbitts and Krizek, 1978). Even chambers specifically designed for gas exchange studies will fall short of the requirements for a CELSS type study where the integrity of the system would have to be maintained for months. Sealing a chamber having multiple ports and utility lines to CELSS quality would require special knowledge and skills. However, with recent advances in space technology and materials, such a CELSS chamber may now be feasible.

The prototype CELSS chamber would have to have the capacity of maintaining the gas ratios in the atmosphere as the plants grow through their life cycle and fill the chamber. Other plant requirements such as temperature, light, water and nutrients will also have to be available while maintaining the integrity of the gas seals. Ethylene, terpenes, CO_2, O_2 and other compounds that are given off by plants will have to be monitored and excesses absorbed or maintained at benign concentrations. In addition to having to meet all of these requirements, the more strigent requirement will be that no materials, solids, liquids or gases, can be added or removed. With such a system, it may be possible to fully understand the

requirements for growing several species of plants singly and simultaneously in a CELSS for a long period of time. Perhaps plant growth systems such as that built by Schwartzkopf and Stofan, 1981, could be used as a starting point for the design of an upgraded second generation system that would fulfill the CELSS requirement.

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A METHOD FOR SCREENING OF PLANT SPECIES FOR SPACE USE

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INTRODUCTION

Higher plants are capable of performing important functions of environmental regeneration and food production in future space or planetary habitats. Also, tending of some plants within a spacecraft or habitat may have psychological and morale benefits to space crews. Success will depend upon the plant's responses to special environmental factors including reduced gravity. For example, plants may be cultured on a large scale in radically altered gaseous environments (e.g., very low pressures) which would offer considerable savings in structural and energy expenditures. However, if plants and other living organisms are rejected as part of future space habitats, it may result not from a lack of biotic capabilities, but from a failure on the part of biologists to demonstrate and express these capabilities in a rigorous, quantitative manner acceptable to space-planning engineers.

Technology transfer between the physical sciences and engineering is accelerated by the common disciplines and languages of thermodynamics, material properties, mathematics, and computer analyses. Conversely, the interface between basic biology and agriculture is highly subjective and slowed by specialized jargon, especially in the new areas of biotechnology. Historically, the quest for more detailed biological information has obscured the need for unifying concepts and theory. Thus while "genetic engineers" are rapidly learning how to modify individual genes, there is difficulty identifying the processes which need to be altered and quantitatively predicting improved performance under specified environmental conditions.

During the past 10 years, progress has been made in the development of "crop models" designed to predict the performance of certain species and cultivars in Earth environments. In the early days, these were primarily "black box" models based on easily measured overt behavior (e.g., growth, flowering, photosynthesis, etc.) fitted to environmental inputs by regression to linear or polynomial equations. These models were highly site specific and thus not easily transferred from one Earth environment to another, much less to space habitats.

Recent modelling efforts, including ours (i.e., Biosystems Research Group) have been more mechanistic, incorpora-

ting quantitative concepts of physics, thermodynamics, biochemical rate kinetics, feedback control, physiology, and anatomy. However, as models become more complex the likelihood of different models predicting similar overall behavior increases. Thus comparison, validation, and/or further development of such models requires experimental techniques capable of simultaneously measuring numerous interactive processes within the modelled organism.

Need for Innovative Experimental Methods. Developing and using plant models to select appropriate organisms for space environments, suggesting genetic improvements (by either conventional or molecular methods), and optimizing plant growth systems will require certain experimental information not fully available at present: (1) We must understand how plants will operate under altered atmospheric pressure; (2) We must optimize in terms of (a) selecting growth conditions within the chosen envelope of possible conditions dictated by structural engineering and operating parameters; and (b) selecting or breeding plant species and varieties most suitable for use under the chosen conditions to meet human nutritional requirements.

The traditional approach to answering such questions is to conduct field or growth chamber tests in which potentially useful varieties are screened against the range of possible conditions and performance is judged, at some end-point, in

terms of health, total growth, fruit yield, etc. Such an approach is time-, labor-, and space-consuming but for standard terrestrial conditions is nonetheless relatively manageable. However, if we consider the necessity of conducting such tests under altered atmosphere and pressure and ultimately perhaps under altered gravity environment, the logistical problems and associated costs become intolerable, if not insurmountable. A means of greatly simplifying and reducing costs of selective research is needed if we are to accomplish the testing necessary to define the plant species and varieties and the conditions under which these plants will be grown.

A second major problem in evaluating plant responses to environmental factors (e.g., temperature, gas composition, nutrient supply, etc.) is in separating the primary effects from secondary feedback and/or adaptive responses. This is critical in the development of predictive, mechanistic models. For example, the concentration of CO_2 directly and immediately affects photosynthesis, plant responses to ethylene, and perhaps a few other metabolic processes. After prolonged exposure, however, other responses appear such as altered carbohydrate allocation, premature senescence of some leaves, and early flowering in some species. The problem then is to determine which of these processes were affected directly by CO_2, and which were induced indirectly by the primary mechanisms.

The best means of discerning primary and secondary mechanisms is continuous, simultaneous measurement of key processes in individual live plants responding to step changes in given environmental factors. Unfortunately, conventional methods for measuring many internal processes require destructive sampling of plant populations at various intervals during an experiment. Such experiments are time consuming and expensive, and provide little assurance that the important dynamic transients and interactions will be observed.

In this paper, we will describe a cost-effective methodology which approaches the rigorous demands of theory by simultaneously monitoring numerous dynamic aspects of carbon assimilation and allocation kinetics in live, intact plants. Analogous methods can apply to nitrogen uptake and allocation.

This methodology capitalizes on the special properties of the short-lived, positron-gamma emitting isotope ^{11}C, especially when applied as $^{11}CO_2$ in a special "extended square wave" (ESW) pattern. As indicated previously (Fares et al., 1978; Magnuson et al., 1982; Goeschl et al, 1984), the 20.4 minute half-life allows for repeated or continuous experiments on the same plant over periods of minutes, hours, days, or weeks. The steady-state isotope equilibrium approached during the ESW experiments, and the parameters which

can be analyzed by this technique are also direct results of that short half-life. Additionally, the paired .511 MeV gamma rays penetrate any amount of tissue and their 180° opposite orientation provides good collimation and allows coincidence counting which nearly eliminates background.

TECHNIQUES

The ^{11}C System. Schematically, the present ^{11}C labelling system operates as depicted in Figure 1. It has three basic components:

1. An accelerator such as a cyclotron or a Van deGraaf accelerator in close proximity to, and capable of providing a continuous stream of radioactive CO_2 to:
2. A precisely controlled plant growth chamber; and
3. A set of radiation detectors and other measuring devices connected to a system of counters and other output devices which are on-line to a computer.

CO_2 is continuously fed into the accelerator target chamber where it is activated to a mixture of gases including ^{11}CO. The radioactive mixture is piped through a chemical conversion system to remove unwanted gases and to convert the ^{11}CO to $^{11}CO_2$. The labelled CO_2 is then mixed in the proper proportions with a standard atmosphere and piped into a clear, plastic photosynthesis chamber attached to the test plant leaf where both $^{12}CO_2$ and $^{11}CO_2$ are taken up by the plant and converted by photosynthesis into carbohydrates. Movement of the ^{11}C-labelled photosynthates through the test plant is monitored by a series of detectors attached at

chosen points on the plant. Various chemical and physical sensors measure system conditions, gas exchange, and other plant responses. That data is collected, stored, and displayed in a computer system to provide real-time experiment monitoring.

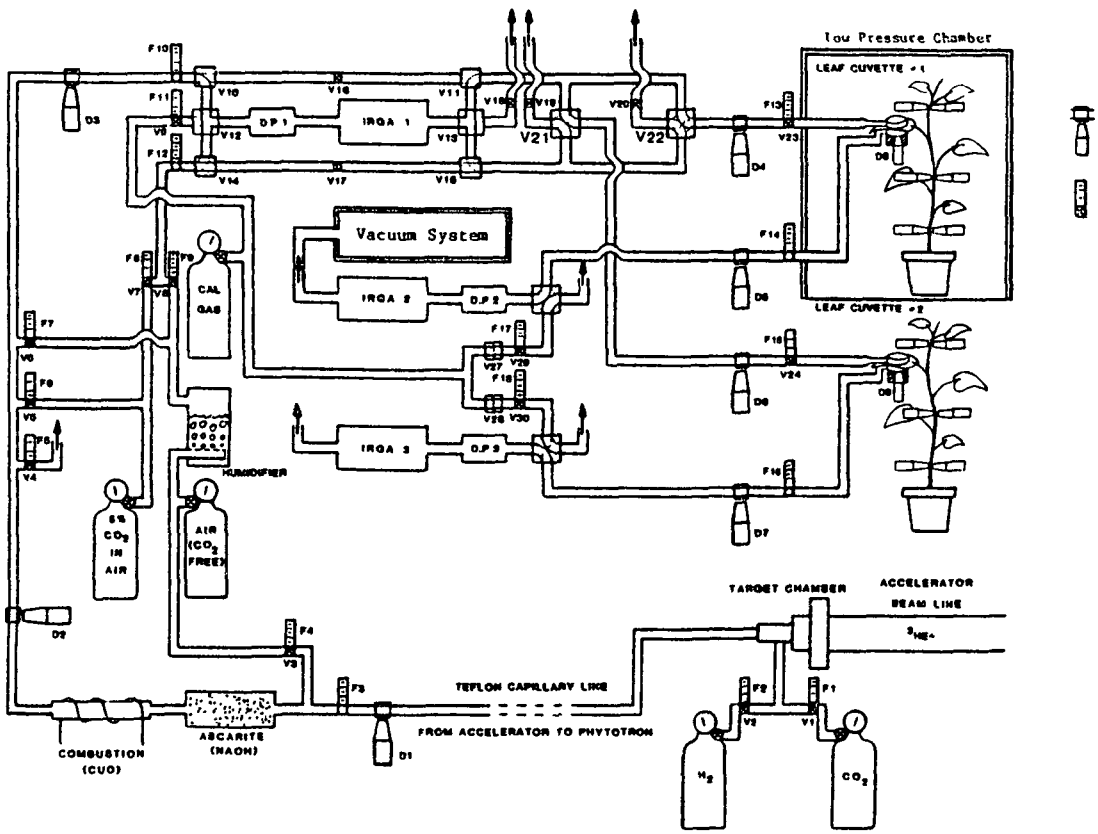

Figure 1. Schematic of the existing ^{11}C System. Lower right - beam line and target chamber; Lower left - chemical processing system; Upper left - gas mixing and measurement; Upper right - plants with leaf chambers and detectors. (Electronics and computer are not shown); Center - output gas measurement and calibration system. The components blocked with heavy (double) lines are the points at which low pressure modification would be made.

The Extended Square Wave. Figure 2 presents typical results of a ^{11}C study of carbon assimilation and allocation in a plant. These data are analyzed by computer along with the gas exchange data. Based on these calculations, the following parameters can be estimated:

1. Transpiration } stomate conductance
2. Leaf Temperature }
3. Net Photosynthesis Rate (CER)
4. Rate of photosynthate storage in the leaf
5. Rate of photosynthate export
6. Export pool "size" or concentration
7. Export pool turnover time
8. Speed of translocation
9. Concentration of translocates
10. Unloading rates at various sinks

EXAMPLES OF RESULTS

Evaluation of Genotypes. Application of the technique to the problem of comparing genotype productivity potentials results in a genetic performance histogram like Figure 3. The histogram pinpoints the genetically controlled limiting property in a particular genotype. In the example shown, each bar represents the ratio of an activity or property of a Mississippi genotype of the C_4 grass Echinochloa crus-galli divided by the same activity or property of a Quebec genotype. The Quebec biotype is adapted to short growing seasons, and thus has a very rapid rate of dry weight accumulation for the first four weeks of growth compared to the

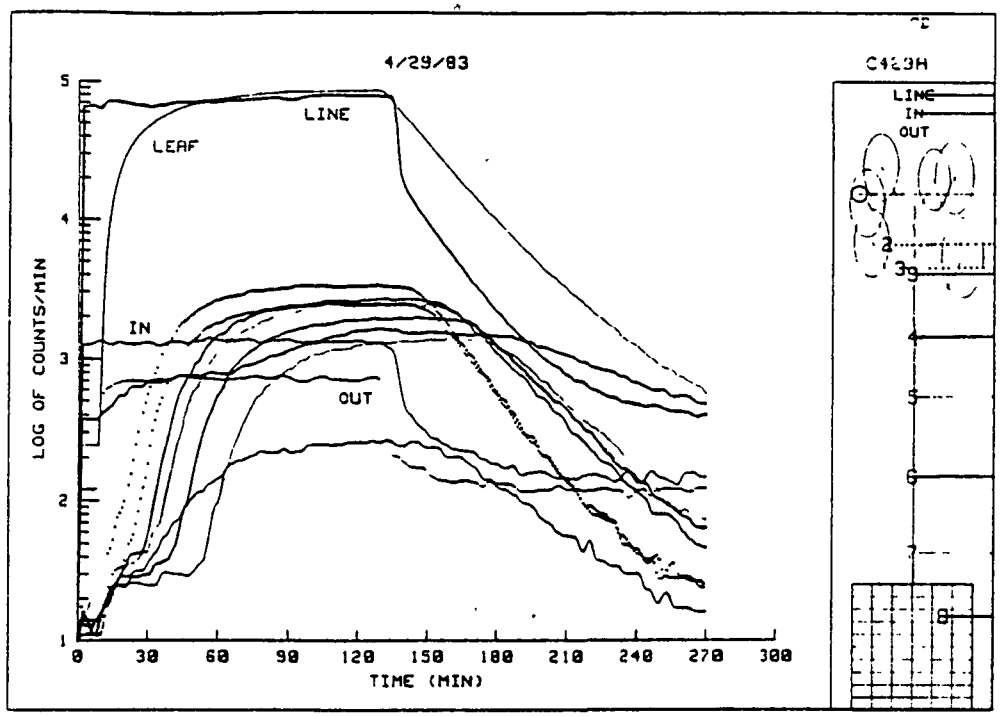

Figure 2. Raw data taken in real-time of a typical ^{11}C metabolism experiment. These traces are stored digitally and recalled for analysis as needed. Each trace represents the calculated ^{11}C concentration at a different position on the plant shown schematically to the right of the trace.

Mississippi biotype (Potvin et al., 1984). The upper five open bars relate growth and net productivity of the genotypes. The remaining open bars represent processes involved in the rate of dry weight gain. The solid bars represent some of the plant's genetically controlled properties which control those processes.

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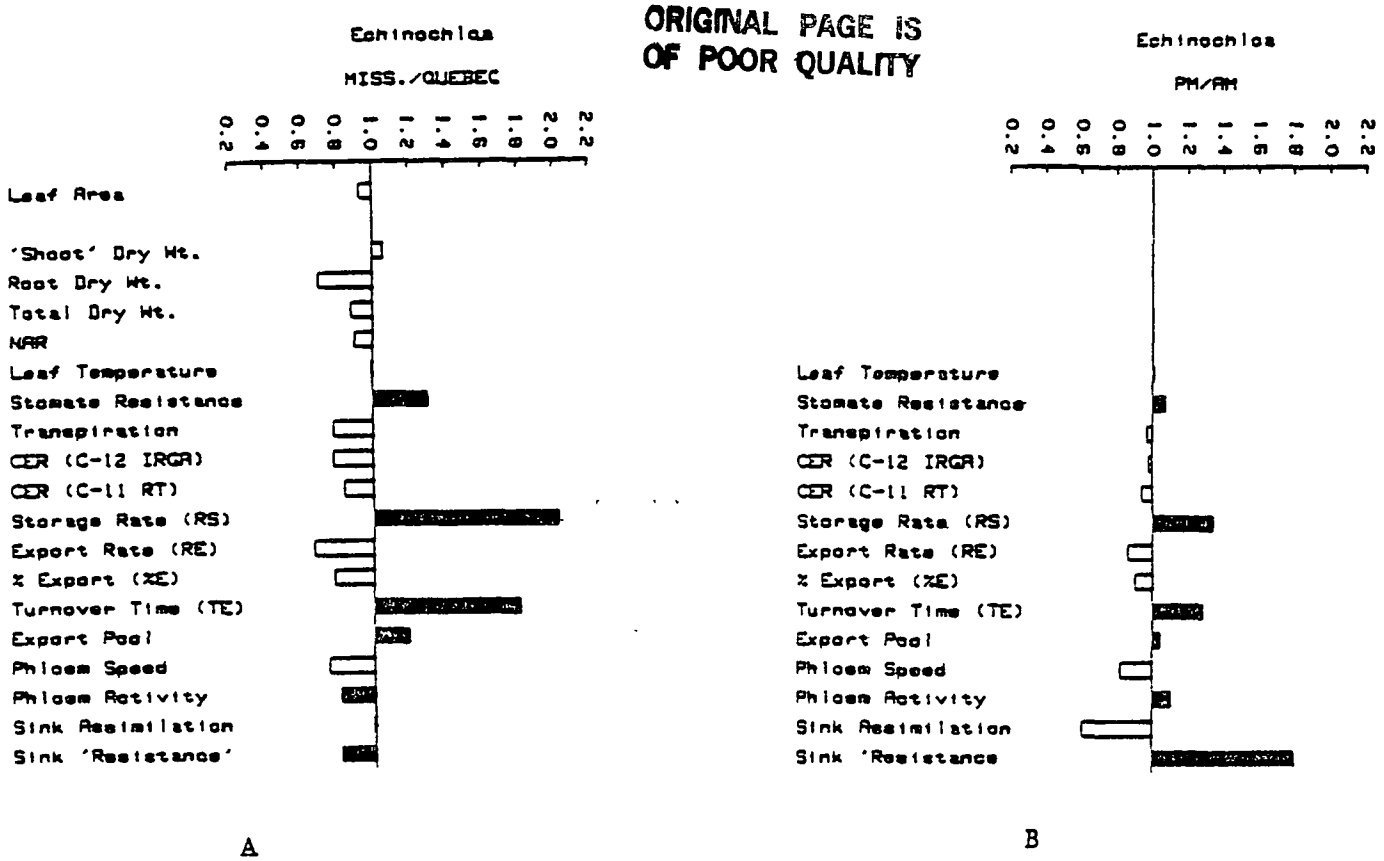

Figure 3. Genetic Performance Histogram (GPH) of cool climate and warm climate biotypes of barnyard grass Echinochloa crus-galli. A - Comparison of performance of the two biotypes; B - Morning versus afternoon performance of the biotypes. Each bar represents an activity or property of the Mississippi biotype divided by the same activity or property of Quebec.

To find the genetically controlled parameters responsible for the lower productivity of the Mississippi genotype, begin at the bottom of the histogram and move upward to the first solid bar to the right of the axis. In this case, that bar represents the concentration of exportable products in the leaf. This suggests that loading of these products into the phloem transport system is a major limitation, as further

reflected in the slow turnover time of these products and the increased rate of storage. If photosynthesis per se were limiting in the Mississippi genotype, one would expect storage and export pools to be lower, i.e., these solid bars would be to the left of the axis.

Sources of variability. Differences in the rates of various processes and the properties which control those processes are often as great between morning and afternoon as they are between genotypes. In Figure 3-b, data are based on the afternoon/morning (PM/AM) behavior (in controlled environment conditions) of the same plants shown in the Mississippi/Quebec comparison (Figure 3-a). Note, particularly, that if only the normal parameters of net photosynthesis (CER), transpiration, and leaf temperature were measured, little difference would be observed.

Other similar sources of variation include recovery of plants from experimental "set-up" procedures, effects of day/night temperatures, or pot size. The important point is that many of the physiological responses would not be detected were it not for the real-time, non destructive nature of the short-lived isotope methods.

CONCLUSIONS

These techniques have been used to observe other phenomena, some of which had not been previously observed, but were predicted from biophysical-thermodynamic theory of

carbon transport in plants (DeMichele et al., 1978; Goeschl et al., 1976; Magnuson et al., 1979). One of these phenomena, the spontaneous blocking and unblocking of phloem transport (Goeschl et al., 1984), was particularly striking when observed with our unique ^{11}C steady-state labelling system. Also, we have recently reported experimental evidence supporting an hypothesized role of the phloem transport system in the response of plants to drought stress (Goeschl et al., 1984; Fares et al., 1984).

The above observations illustrate the potential capabilities of the short-lived isotope labelling techniques as means to (1) evaluate the performance of plants in specialized environments; (2) distinguish and quantify primary environmental effects and secondary physiological adaptations; and (3) provide validation and further development of predictive, mechanistic plant models for use in the engineering design and management of space habitability systems.

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POTATO LEAF EXPLANTS AS A SPACEFLIGHT PLANT TEST SYSTEM

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The advent of regular flights of the Space Shuttle has provided an excellent opportunity for scientists to examine the effects of near weightlessness on plant growth and development. However testing during shuttle flights is faced with significant constraints. Among the more obvious of these constraints are: 1) the relatively short duration of shuttle flights, i.e., the limited time in microgravity, 2) limitations on the size of the test package, and 3) difficulties in maintaining a high degree of environmental control. The first constraint is relatively fixed, while the latter two have some degree of flexibility but are limited from a practical standpoint of cost and electrical power requirements to run experiment growth chambers; in particular, the power constraint could severely limit levels of cooling control and irradiation for photosynthesis. These impasses can be circumvented if plant materials and experiments are chosen accordingly, e.g. the seedling experiments of Cowles et al. (1984) and Brown and Chapman (1984). Unfortunately, from a perspective of developing a higher-plant "CELSS" for long duration spaceflight, it may be difficult to extrapolate results from seedling studies to mature, whole-plant phenomena such as flowering, fruiting, tuber, and tuberous root development (Wheeler and Tibbitts,

1984). Yet to evaluate and justify further ground-based testing of potential crops for spaceflight, it would be beneficial to gather information on the progression of advanced plant development under microgravity as soon as possible.

Clearly it will be impossible to grow candidate food crops through a full life cycle during Shuttle flights. However use of excised tissue or organs from Earth-grown plants may provide an alternate approach to study advanced stages of plant growth and development. For such an approach, it is important to bear in mind that initial development of tissue occurs under a 1-g environment, and these effects would have to be taken into account with appropriate controls. Whether the plant tissues sustain a "memory" of 1-g environment that affects later development is a not known and solving this likely will come only when full-term growth studies can be conducted.

The potato, Solanum tuberosum L., is one of several food crops selected for early investigation for CELSS studies (Tibbitts and Alford, 1982), and it has been known for many years that axillary buds of potato leaves have the ability to develop into a variety of stem forms (Vöchting, 1887). By excising a potato leaf and its subtended axillary bud and maintaining it in a humid environment, the bud can

be forced to develop rapidly (Gregory, 1956; Ewing, 1978). The growth form expressed by the bud appears to be directly controlled by inductive state of the mother plants (Ewing, 1978); namely, leaf cuttings from induced plants (i.e., plants that were grown in an environment promotive of tuber formation) will produce tubers at the axillary bud; in contrast, cuttings from non-induced plants tend to produce leafy upright shoots, while intermediate states of induction result in intermediate responses such as stolons (horizontal, leafless stems) or elongation tubers (Fig. 1).

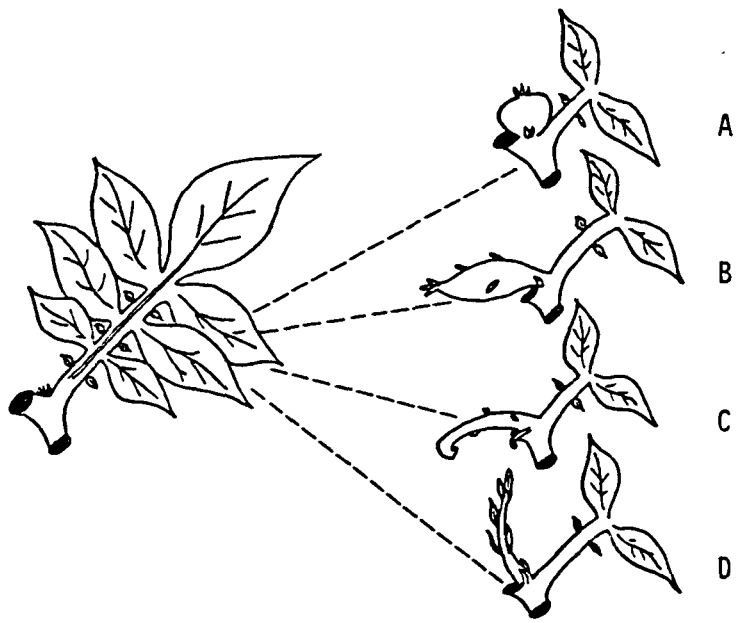

Figure 1. Drawings of axillary bud development of potato leaves 10-14 days after excision. (A) round sessile tuber on cutting taken from a plant induced to tuberize; (B) elongated tuber and (C) stolon from partially induced plants; (D) upright leafy shoot from non-induced plant.

Studies using excised potato leaves commonly involve placing the cuttings in mist beds or humidity chambers with

the basal cut end buried in a moist growing medium (Kahn et al., 1983). The buried buds develop into discernible growth forms within 4-5 days of excision, although 10 to 14-day growth cycles are frequently used to obtain full development. Nearly all leaves are capable of producing axillary bud growth after excision, but young, fully expanded are most effective (Kahn et al., 1983).

By knowing that the bud growth of leaf cuttings is controlled by the degree of tuber induction in the mother plant, one can control the leaf cutting response by controlling the growth of the mother plant. For example, tuberization of potato is known to be promoted by short photoperiods, cool temperatures, and high irradiance levels (Gregory, 1956; Ewing, 1978), thus growing plants under these conditions will lead to leaf cuttings which consistently yield tubers. Extending the photoperiod, particularly with dim light, shifts the cutting response toward leafy shoots. Similarly, increasing the temperatures (e.g. > 20^{o}C) shifts the response toward leafy shoots, particularly in combination with long photoperiods. Hence the potential exists for using potato leaf cuttings to test a variety of stem growth phenomena.

Cuttings that form sessile tubers in the leaf axils tend to produce the most biomass during 10 to 14-day growth

cycles (Kahn et al., 1983). Starch deposition and cell division increase rapidly in the buds following excision, while proteins specific to tubers can be detected within 48 h (Duncan and Ewing, 1984; Paiva et al., 1983). Thus the enlarging tubers at the leaf axils appear to be physiologically and anatomically similar to tubers formed on stolons of intact plants (Duncan and Ewing, 1984; Paiva et al. 1983).

Leaf cuttings in the PGU to study tuber growth. The size of potato leaves should pose little or no problem with regard to fitting small growth chambers such as the 1-liter PGC's of NASA's plant growth unit (PGU). Young, near-fully to fully expanded leaves can range from 10 to 20 cm length, depending on the cultivar and growing conditions. As with tuber induction, the size of potato leaves also can be controlled by environmental factors; therefore, it should be possible to selectively grow and choose leaves which maximize the available volume of the growth chambers. This may be an important consideration for maximizing total growth of the leaf cutting bud which appears to vary directly with leaf area (Kahn et al., 1983).

Typically, tuber formation is not visible until 3 to 4 days after excision but enlargement then proceeds rapidly (Fig. 2). To date, shuttle flights have averaged 7 days $\pm$ 2

days (Halstead and Dutcher, 1984) indicating approximately 0.5 g of tuber fresh mass could be obtained during an average shuttle flight (Fig. 2). The yields could be substantially increased (to 1.0 to 2.0 g) by excising leaves 4 to 5 days prior to launch thereby shifting the final harvest to 11 or 12-days-age. In this case, the rapid growth stage of bud development could be studied during microgravity.

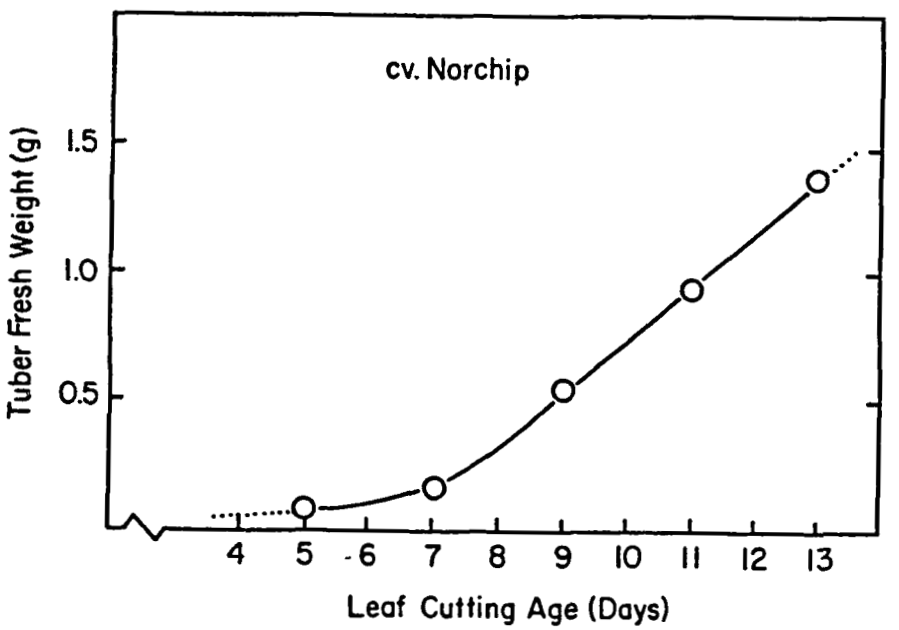

Figure 2. Growth of tubers in axils of potato leaf cuttings.

Leaf cuttings grown in humid environments for 14 days can show up to 50% increases in total fresh mass, most of which can be accounted for strictly by bud growth. This indicates that bud growth is driven by photosynthesis rather than a reallocation of existing leaf carbohydrate. Thus

irradiance levels may be a limiting factor for bud growth. To test this, cuttings from induced plants were grown under different irradiance levels obtained with varying amounts of neutral white shading. After 14 days, cuttings grown under 225 μmol $s^{-1}m^{-2}$, a level similar to that used in our past studies, produced tubers averaging 2.3 g fresh mass; in comparison, cuttings grown under 75 μmol $s^{-1}m^{-2}$, a level similar to that produced by the fluorescent lamps in the PGU, averaged nearly 2.0 g fresh mass. It appears then that bud weight gains can be enhanced by increasing the irradiance level, but good tuber growth can be obtained under 75 μmol $s^{-1}m^{-2}$. Therefore, the light levels available in a growth module such as the PGU should not be limiting for potato leaf cutting growth.

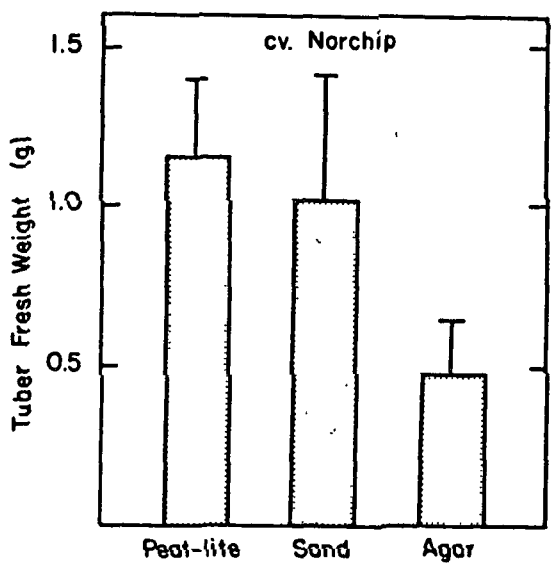

Figure 3. Comparison of tuber growth on 14-day-old potato leaf cuttings grown in peat-vermiculite (50:50 v/v), sand, and 0.6% agar. Standard deviations are shown.

Solid media such as sand or a peat-vermiculite mixture (50:50 v/v) sustain good leaf cutting growth, while a 0.6% agar medium does not (Fig. 3). The 'peat-lite' medium is light weight and has a high water holding capacity; also, this medium has been used effectively for spaceflight tests with sunflower seedlings (Brown and Chapman, 1984).

Further testing with leaf cuttings. Conceivably potato leaf buds could be grown in sterile-culture vessels on a sugar-supplemented medium thereby eliminating the need for the attached leaf and greatly reducing the overall size (Gregory, 1956; Ewing and Senesac, 1981). This approach would require complete surface sterilization of the test materials but the reduced size would permit increased sample numbers and easier accommodation to further experimental manipulation, such as spaceflight centrifugation.

The utility of potato leaf cuttings as a plant test system need not be confined strictly to the study of potato tuber (or other stem form) growth and development. The vitality and persistence of these explants makes them excellent candidates for potential photosynthesis, respiration, or other gas exchange studies as well as simplified source-sink models for carbohydrate translocation and metabolism experiments.

Summary. The use of explant tissues or organs may circumvent limitations facing whole-plant experimentation during spaceflight. In the case of potato, a crop currently being studied for application to bioregenerative life support systems, excised leaves and their subtended axillary buds can be used to test a variety of stem growth and development phases ranging from tubers through stolons (horizontal stems) to upright leafy shoots. The leaves can be fit well into small-volume test packages and sustained under relatively low irradiance levels using light-weight growing media. Tubers formed on potato leaf cuttings can yield up from 0.5 to 1.0 g fresh mass 10 days after excision and up to 2.0 g or more, 14 days from excision.

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THE EFFECT OF ULTRADIAN AND ORBITAL CYCLES ON PLANT GROWTH

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ABSTRACT

All life on earth has developed under the 24 hr light dark cycle dictated by the earth's daily rotation. There is little information on the degree to which plant growth is obligated to this 24 hr cycle nor the effect of short cycle length on growth. In a series of experiments using sugar beets, we investigated the effects of varying cycles lengths on growth (0.37 hr to 48 hr). Each cycle was equally divided into a light and dark period so that each treatment regardless of cycle length received the same amount of light over the 17 weeks of the experiment. We used two growth parameters to evaluate the effects of cycle length, total fresh weight and sucrose content of the storage root. Both parameters showed very similar responses in that under long cycles (12 hr or greater) growth was "normal", whereas plants growing under shorter cycle periods were progressively inhibited. Minimum growth occurred at a cycle period of 0.75 hr. The yield at the 0.75 hr cycle, where was at a minimum, for total fresh weight was only 51 percent compared to the 24 hr cycle. The yield of sucrose was even more reduced at 41 percent of the 24 hr cycle.

INTRODUCTION

In recent years , much effort has been put forth to learn how to grow plants in a controlled ecological life support system (CELSS) type environment (Wheeler and Tibbitts, 1984). These efforts are reflected in the Total a large number of NASA-sponsored workshops and symposia (Tibbitts and Alford, 1980; Moore et al.,1982; Fabricant, 1983) that have been and are planned to be held, and in the increasing number of papers published on this subject. Generally, the research has been directed toward the selection of candidate species, cultural methods and strategies to maximize food production in the minimum of time and space. Attention is now turning to concepts of how to maximize production per unit of energy used.Thus lighting becomes of concern due to its high energy demands. The energy requirement of light for photosynthesis can be managed through the manipulation of intensity, duration and integration time wise with other energy requiring functions.

The proposed space station in low earth orbit will be in sunlight for approximately one hour and darkness 30 min. of each orbit, i.e. and orbital photoperiod. Thus the available power from solar panels or direct sunlight will cycle on and off every 90 min. This means that any horticultural system growing plants on a 24 hr cycle will require engineering support. The question which arises from this is whether from a horticultural point of view there are significant benefits

from growing on a 24 hr cycle or is it possible to achieve adequate plant production independent of cycle length. Because of the orbital photoperiod which the proposed space station will occupy it is desirable to known if sugar beet, a plant selected for CELSS (Tibbitts and Alford 1980) is affected by cycle length.

METHODS AND MATERIALS

Ten seedballs of sugarbeet (Beta vulgaris L.), equally spaced, were placed in a circle 12 cm in diameter, and planted to a depth of 2.0 cm in No. 2 vermiculite contained in 20-liter pots lined with 1.5-ml polyethylene liners. The seedlings as they grew were gradually thinned to two plants per pot. The plants were watered daily with one-half strength Hoaglands nutrient solution (Hoagland and Arnon 1950) modified to include 0.5 mM NaCl (Ulrich et al. 1958). Plants from the time of planting to harvest were grown in controlled environment chambers (127 X 249 X 137 cm.). The temperature was a constant 20 degrees C and illumination was 650 uE/cm2/sec of photosynthetically active radiation supplied 80% by cool-white VHO fluorescent lamps and 20% by incandescent lamps.The relative humidity was not controlled but was approximately 70% ambient CO2 was used with about 20% make up air. A single growth chamber was used for each treatment. At the start of the experiment each chamber contained thirty-two 20-liter pots placed in a 4-row by 8-column pattern. Each harvest consisted of eight plants with

two plants taken from each column. At the final harvest each chamber had a single row of eight pots along the center of the chamber. Plants were harvested at 5, 9, 13 and 17 weeks after planting. Fresh weights of tops and storage roots were taken on a pot basis and dried in a 70 degree oven for dry weights. Samples of pulp from the storage root were analyzed for sucrose.

RESULTS AND DISCUSSION

Figure 1 shows total yield fresh weight as a function of cycle length (cycle length in this paper is expressed as the sum (hr) of a consecutive light and dark period).

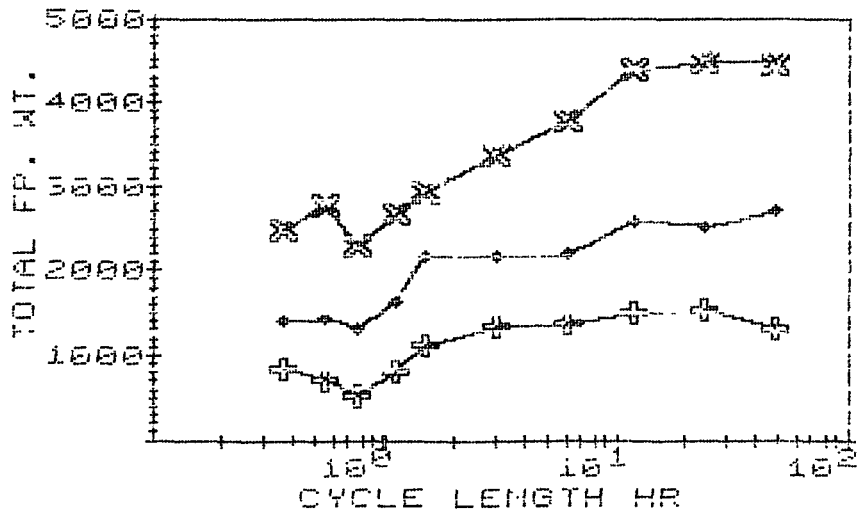

FIGURE 1 The total fresh weight per pot including the storage root (average of 8 pots/2 plants each in grams). (● = 9 weeks; + = 13 weeks; X = 17 weeks)

The three curves show the total fresh weight for harvests at 9, 13 and 17 weeks as a function of cycle time. The curve depicting the 9 week harvest for total fresh weight clearly shows that at a cycle time of 0.75 hr there occurs a minimum in yield. Yield increases at both longer and shorter cycles times. However the largest yields are always associsted with the longer cycle times. The 17 week yield curve also shows a minimum at 0.75 hr cycle time. However in this curve the difference in yield between the longer and shorter cycle times is much more pronounced. This seems to indicate that as the plant ages, that the longer cycle times become more important in relation to yield. The importance of the yield minimum at 0.75 hr may only be in its relationship to the mechanism of action of this cycle phenomena. The response of the 13 week harvest as would be expected is somewhat intermediate between the other two harvests.

The curves in figure 1are a composite of two experiments run at different times in a single set of growth chambers one experiment covered cycle times from 0.37 to 1.5 hr and the other from 1.5 to 48 hr.. Although the environmental parameters and cultural practices were designed to be identical for both experiments. The experiment with the longer cycle treatments did show differences in yield in the overlapping treatment at 1.5 hr cycle time. This is most clearly shown in the 17 week harvest by an offset in the graph at the 1.5 hr cycle time where the two experiments have an

overlapping treatment(figure 2).

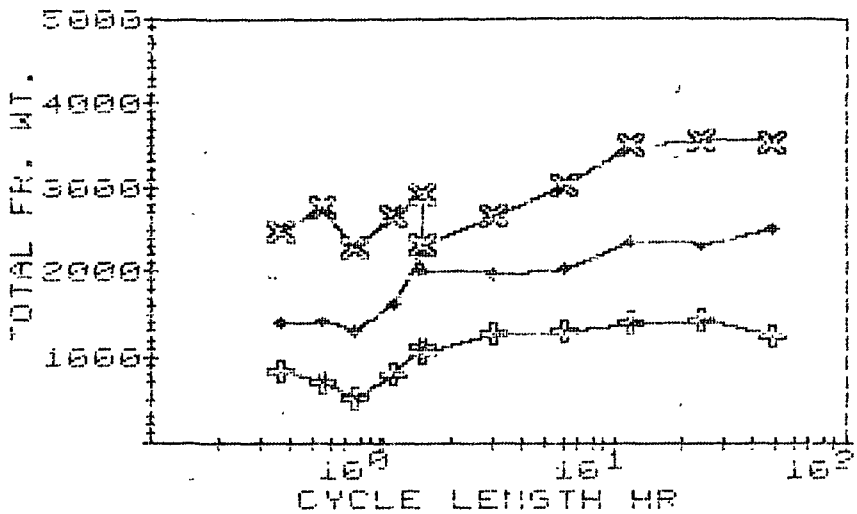

FIGURE 2 Total fresh weight without normalization of the data from the experiments with the longer cycle lengths,1.5 to 48 hr.
(✥ = 9 weeks; + = 13 weeks; X = 17 weeks)

This difference between experiments, run at different times but in the same set of growth chambers, in the nonnormalized curves does provide additional evidence that common cultural practices, although they do affect yield, do not seem to affect the observed responses related to cycle time.

The yield differences shown in figure 1 are not small in that even a conservative estimate indicates a yield of 50 to 30 percent for weeks 9 and 17. Thus if the growth differences are calculated for the 9 week harvests for the longer cycles (12 to 48 hr) and compared with the 0.75 hr cycle plants (45 min) there is a difference of 66 percent. When calculated for the 17 week harvest the yield difference is decreased but is still 50 percent. Similar cycle treatments to buckwheat and cosmos by Garner and Allard (1931) showed an even greater

yield difference, in some cases as much as 88 fold difference. The yield curves for the three different harvest dates all show the same general shape curve in that there is a minimum at the 0.75 hr cycle time with higher yields at both shorter an longer cycle times. However the size of the minimum at 0.75 hr is less at the later harvests. The high degree of parallelism between the curves for these three harvests is strong evidence that this phenomena is related to the variable of this experiment, cycle time rather than some specific aspect of a certain phase of growth and development. These sugar beets at the time of the 17 week harvest were large plants, thus these plants have passed through all the growth stages up to flowering.

Sugar beets are rather unique in that, in addition to total biomass being a good index of yield, the percent sucrose in the storage root is an excellent index of the amount of photosynthate available to the plant in excess of its needs for immediate growth. Thus if the concentration of sucrose is graphed as a function of cycle time it should provide a independent evaluation of the effects of cycle time on photosynthesis versus other aspects of plant growth and development. Figure 3 shows this graph where there is also a minimum in sucrose concentration at the 0.75 hr cycle time which continues up to the 13 week harvest.

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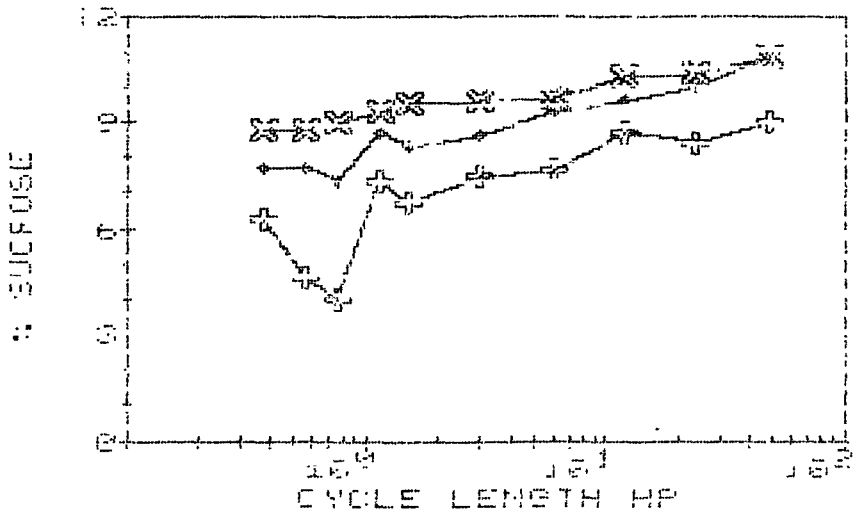

FIGURE 3 Sucrose percentage in the storage root. Each harvest curve is the average of 8 pots/ 2 plants each. (● = 9 weeks; + = 13 weeks; X = 17 weeks)

This minimum in sucrose concentration occurs in conjunction with a minimum in biomass. If the photosynthetic capacity of the plant had remained the same, then in the presence of reduced growth as occurred under the 0.75 hr cycle it would be expected that the sucrose concentration would have risen. However as seen in figure 3 there is also a minimum in sucrose concentration. This reduction in sucrose concentration has to be related to a greater effect of the cycle treatment on the photosynthetic mechanism than the rest of the plants growth and development.

However at the 17 week harvest there does not appear to be a minimum but rather a constant decline in sucrose

concentration with decreasing cycle time. This seems to imply that at the 0.75 hr cycle time there is a minimum in the amount of photosynthate available for growth. All plants in these experiments received the same amount of total light the only difference being cycle length. Thus the observed difference can not be related to differences in the amount of light received but only to differences in the plants ability to utilize this light. The work of Bonde (1955) showed a minimum in chlorophyll content in the leaves of cocklebur under cycle times less than an hour. He also indicated that the chlorophyll decrease was equally noticeable at three light intensities 500, 1000, and 1500 FC. However he reached the conclusion that yield reduction was not due to effects on the photosynthetic mechanism because sucrose sprays had no offsetting effects on yield reduction (Bonde 1956). Unfortunately he did not determine if in fact the sucrose was absorbed by the plant. The change in the characteristics of the curve at the 17 week harvest in (figure 3) to just a yield decrease at shorter cycles times rather than the clear display of a minimum at 0.75 hr seems to suggest that there is a degree of adaptation with age to at least one aspect of the reduction in photosynthate. The consistent decrease of sucrose percentage with decreasing cycle length compared with the break in slope (at the 12 hr cycle length of the total fresh weight in the 17 week harvest (figure 1) would suggest that with increasing age longer cycle lengths may be more

favorable.

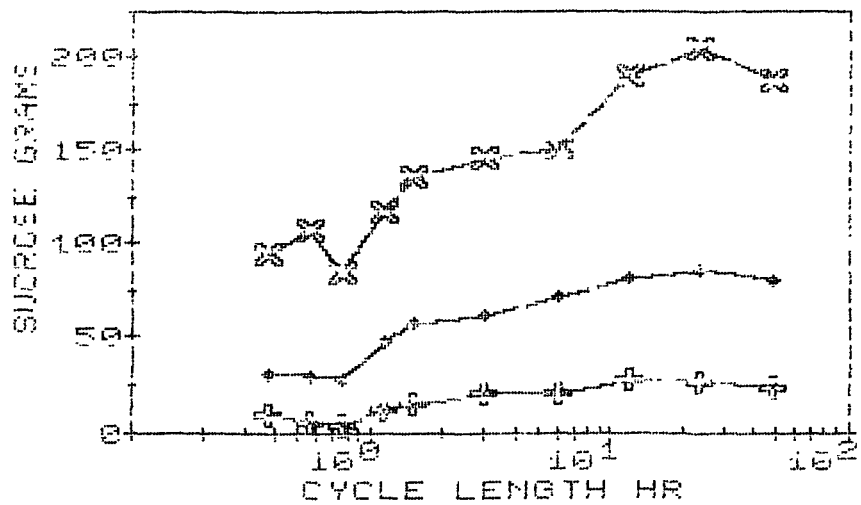

FIGURE 4 Total sucrose per pot. Each harvest curve is the average of 8 pots/ 2 plants each. (◆ = 9 weeks; + = 13 weeks; X = 17 weeks)

The amount of sucrose accumulated in the root as a function of cycle length and harvest date is shown in figure 4. This aspect of yield also shows the same general trend in that increased yield is definitely associated with longer cycle lengths, 12 hr or longer. The higher yield at the shorter cycles compared to the 0.75 hr cycle could be a very important factor in determining the mechanism of action but there is still a 30 percent decrease in comparison to the longer cycles. This seems to indicate that the longer cycle times will be necessary for the higher growth rates. These observed responses to cycle length is not predictable from standard text book plant physiological principles.The experiments reported here show that the plants response to

differences in the light dark cycle length even though the total light recieved during the duration of the experiment was identical.

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NITROGEN UPTAKE AND UTILIZATION BY INTACT PLANTS

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INTRODUCTION

The overall objective of the research effort at North Carolina State University is to continue development of a dynamic plant growth model that is capable of simulating dry matter productivity and distribution in response to environmental conditions. We have reported progress in model development in a previous paper in this volume. Inherent in mechanistic models that describe the dynamics of plant growth should be the interrelationship between root function of supplying nitrogen and the shoot function of supplying photosynthate. We have proposed a conceptual model (Figure 1) which describes nitrogen uptake in plants as a function of the balance between root and shoot activities (Raper et al., 1976, 1977, 1978). According to this model, nitrogen uptake is regulated by the balance between the demand for carbon and nitrogen products within the various plant parts, and thus the subsequent balancing of nitrogen flux into the shoot and carbohydrate flux into the root. Since absorption of nitrogen by roots is an active process that requires metabolic activity, nitrogen uptake is responsive to level of soluble carbohydrate in the root (Raper et al., 1978). Uninterrupted uptake of nitrogen by roots, which are inherently low in soluble carbohydrate (Raper et al., 1976, 1978), thus is dependent upon concurrent translocation of soluble carbohydrate from the shoot.

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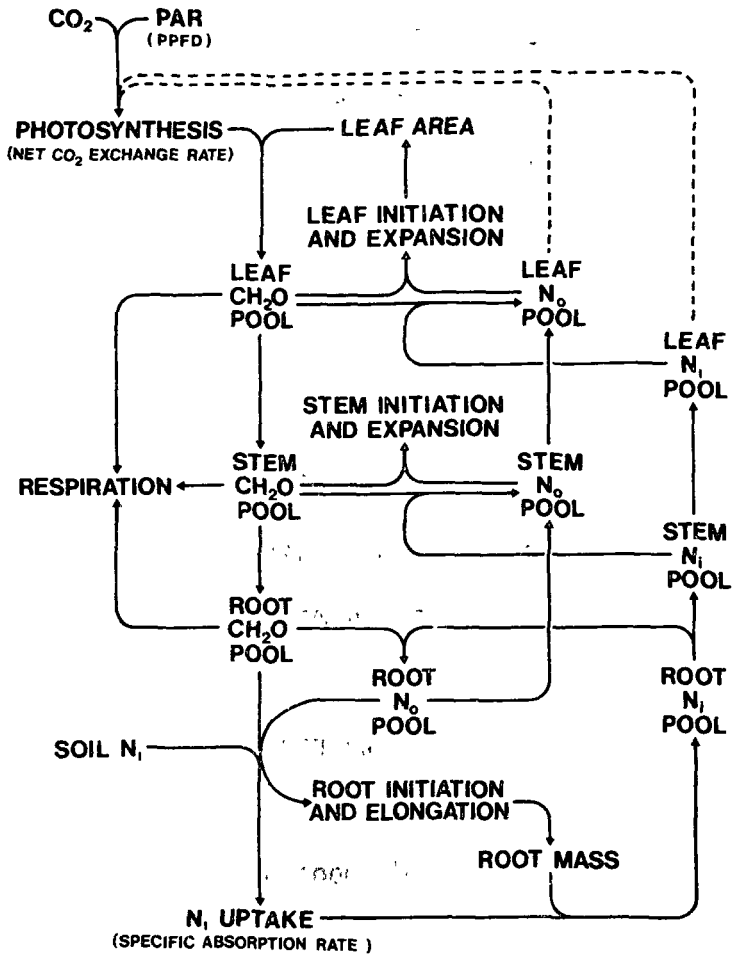


Figure 1. Scheme for balancing the nitrogen-supplying function of roots with the carbon-supplying function of shoots. N_i and N_o indicate inorganic ions (NO_3^- or NH_4^+) and organic products of nitrogen assimilation.

An assumption in the model is that when photosynthate in the shoot (leaves and stems) is limited, it is partitioned within the plant according to the scheme of Thornley (1976). The carbohydrate pool in the shoot is supplied by photosynthesis and is utilized as the source for both growth and respiration within the shoot and as the source for the root pool (Figure 1). Subsequent translocation of carbohydrate is responsive to the concentration of carbohydrate

in shoot pools and size and metabolic activity of sink pools (Wann and Raper, 1979, 1984). As nitrogen absorbed by roots is translocated to shoots, it stimulates initiation and expansion of new leaves (Raper and Peedin, 1978; Rufty et al., 1984). Nitrogen-stimulated metabolic demand of new leaves reduces availability of carbohydrate in the shoot pool for translocation to roots. Since nitrogen uptake is dependent on translocation of carbohydrate from shoot to roots, this model would predict that decreased translocation to roots would reduce nitrogen uptake and, ultimately, amount of nitrogen translocated to shoots. A subsequent reduction in initiation and expansion of new leaf tissue in response to decreased translocation of nitrogen (Raper and Peedin, 1978; Rufty et al., 1984) would reduce shoot demand for carbohydrate before reducing canopy photosynthetic rate (Raper and Peedin, 1978) and, thus, increase availability of carbohydrate for transport to roots. Thus, uptake of nitrogen and partitioning of carbon and nitrogen within the plant are regulated to maintain a functional balance between root and shoot growth.

Four inferences about nitrogen uptake can be drawn from this model for whole plant regulation of nitrogen uptake. First, when grown under near optimum conditions, a fluctuation should occur in the rate of nitrogen uptake which would be a function of the fluctuation in demand of carbon and nitrogen in the shoot and availability of carbohydrate within the roots to support the uptake process. Second, if root function is disturbed, an alteration should occur in rate of nitrogen uptake which might also be associated with a change in pattern of uptake as the plant establishes a new balance between root and shoot function. Third, if external nitrogen supply for the roots is discontinued, initial shifts in dry matter and nitrogen partitioning within the

plant should include a rapid decline in nitrogen content of shoots, with little immediate reduction in photosynthetic rate, and an increase in root dry weight. As the nitrogen stress continues and the nitrogen content of leaves fall below critical levels, photosynthetic rate should drop abruptly and dry weights of roots should decline relative to nonstressed plants. Fourth, if the balance between carbon and nitrogen supplies remains tightly coupled during vegetative growth, there should be little distinction in the utilization of ammonium and nitrate as sources of nitrogen if pH of the nutrient solution is controlled. We have designed experiments to test the validity of each of these inferences as a challenge to the concept of regulation of nitrogen uptake at the whole plant level by the interdependence of root and shoot functionality.

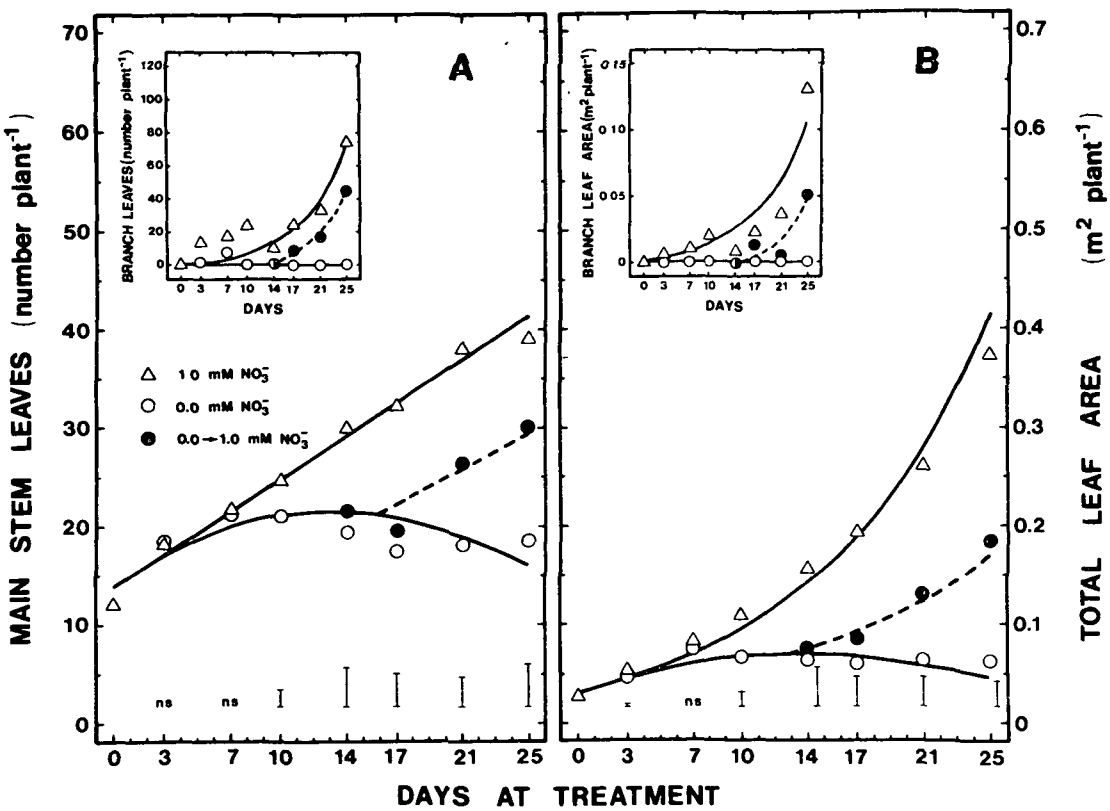
EXPERIMENTAL SYSTEM

In our experiments we use a walk-in growth room of the Phytotron at North Carolina State University (Downs and Thomas, 1983) which has a growing area of 8.92 m^2 and a height of 2.13 m between floor and light barrier. Aerial temperature is monitored and controlled within the growth room to within 0.3 C over an operational range of 7 to 40 C. A combination of cool white fluorescent and incandescent lamps, at an input wattage ratio of 10:3, provide a photosynthetic photon flux density (PPFD) of up to 750 µmol s^{-1} m^{-2} between wavelengths of 400 and 700 nm and photomorphogenic radiation (PR) of 10 w m^{-2} between wavelengths of 700 and 850 nm. Ambient CO_2 is monitored and maintained with injection of commercial grade gas.

A continuous-flow, hydroponic culture system has been constructed to operate within the growth room. The system includes four independent units with continuous monitoring and control of pH (±0.05 pH unit) and temperature (±0.2 C) of the solution. Each of the four units consists of an upper compartment where the plant root systems are suspended in 100 L of nutrient solution and a lower reservoir compartment containing 100 L of solution. Temperature and pH monitoring and control occurs in the reservoir compartment, and the nutrient solution is continuously circulated between the upper and lower compartments at 0.38 L s^{-1}. The upper compartment is divided into 12 8.3-L chambers with individual supply and return lines with the common reservoir. Each of the 12 root chambers per unit can contain one to four plants.

A Dionex Ion Chromatograph system 2110 with dual cation and anion columns is located adjacent to the growth room. From a single injection, anions plus monovalent cations in a sample of nutrient solution can be determined in less than 8 minutes. (With substitution of a new cation column being developed for Dionex, both monovalent and divalent cations can be separated in one column simultaneously.) Sampling and injection currently is done manually, but in the future the chromatograph will be connected on-line with the hydroponics system for automated sampling. Nutrient uptake rates and total nutrient accumulation by plants can be determined by depletion from the nutrient solution. Nutrient concentrations in solutions are replenished to treatment levels by additions of salts in response to depletion during a sampling interval of one day or less.

Figure 2. Effect of nitrogen stress and restoration of nitrogen availability on (A) number of mainstem and branch (inset) leaves and (B) canopy leaf area of soybean.

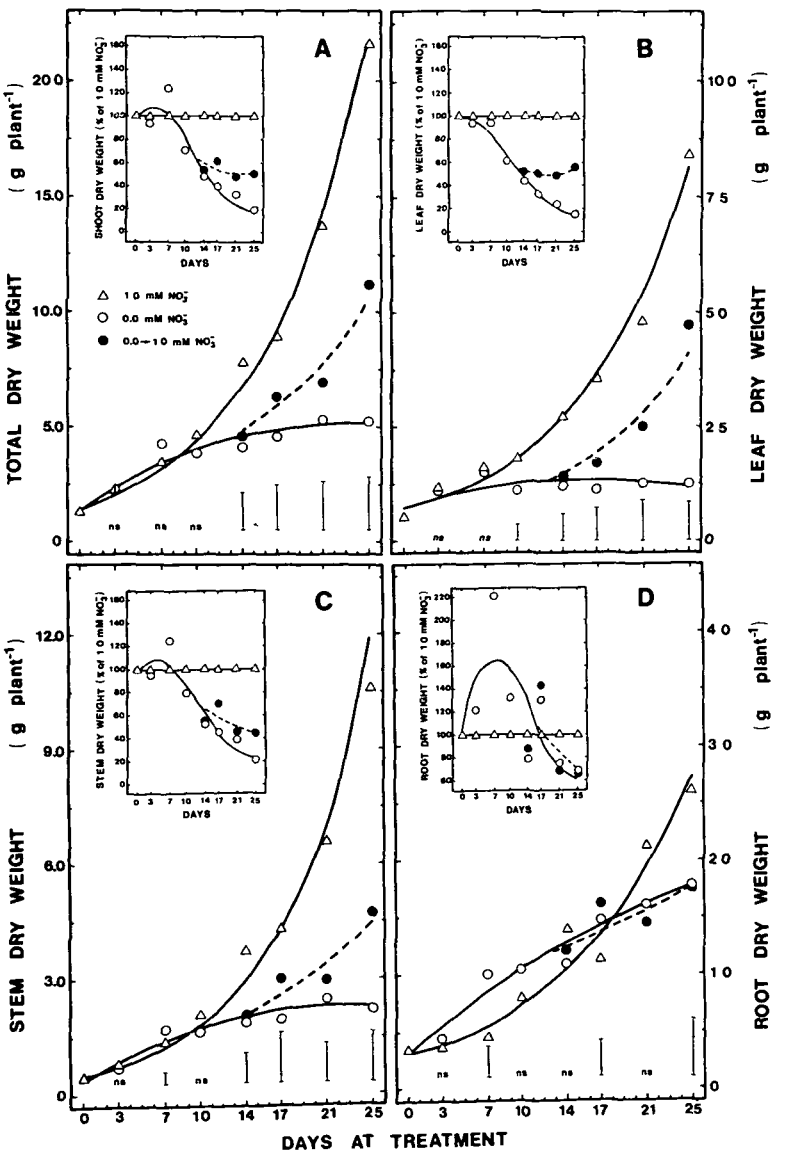


ONSET AND RECOVERY OF NITROGEN STRESS

Nonnodulated soybean (Glycine max (L.) Merrill 'Ransom') plants were grown hydroponically for 14 days with 1.0 mM NO_3^- in a complete nutrient solution. Plants then were transferred to complete nutrient solution with 1.0 mM NO_3^- for 25 days, to a minus-nitrogen solution (0.0 mM NO_3^-) for 25 days, or to the 0.0 mM NO_3^- solution for 10 days followed by transfer to the 1.0 mM NO_3^- solution for 15 days. Throughout the experiment, day/night aerial temperature was 24 C. PPFD during the 9-h day was 700 μmol s^{-1} m^{-2}, and a 3-h interruption of the dark period by incandescent lamps was used to repress floral initiation. Ambient CO_2 concentration was 400 μL L^{-1}. The pH of the nutrient solution was maintained at 6.0.

When NO_3^- was removed from solution, NO_3^- storage pools in the plant were reduced rapidly (data not shown). As nitrogen became limiting for sustained growth and meristematic activity, initiation of both mainstem and branch leaves ceased within 7 days, along with expansion of canopy leaf area (Figure 2). In contrast, root growth of the nitrogen-stressed plants during this first week was increased relative to that of nonstressed plants (Figure 3), and thereafter was reduced as the nitrogen stress continued. Thus, under long-term nitrogen stress, there was an alteration in carbon and nitrogen partitioning predicted by the model (Figure 1). While nitrogen stress reduced the photosynthetic rate of leaves (Figure 4), it resulted in a greater reduction in the initiation and expansion of leaves (Figure 2). As a consequence, the activity of leaves as a sink for photosynthate was reduced more than their activity as a source. Until the total photosynthetic area

Figure 3. Effect of nitrogen stress and restoration of nitrogen availability on dry matter accumulation in (A) whole plants, (B) leaves, (C) stems, and (D) roots of soybean plants. Insets show dry weights of (A) shoot, (B) leaves, (C) stems, and (D) roots of nitrogen-stressed plants as percentages of dry weights of nonstressed plants.



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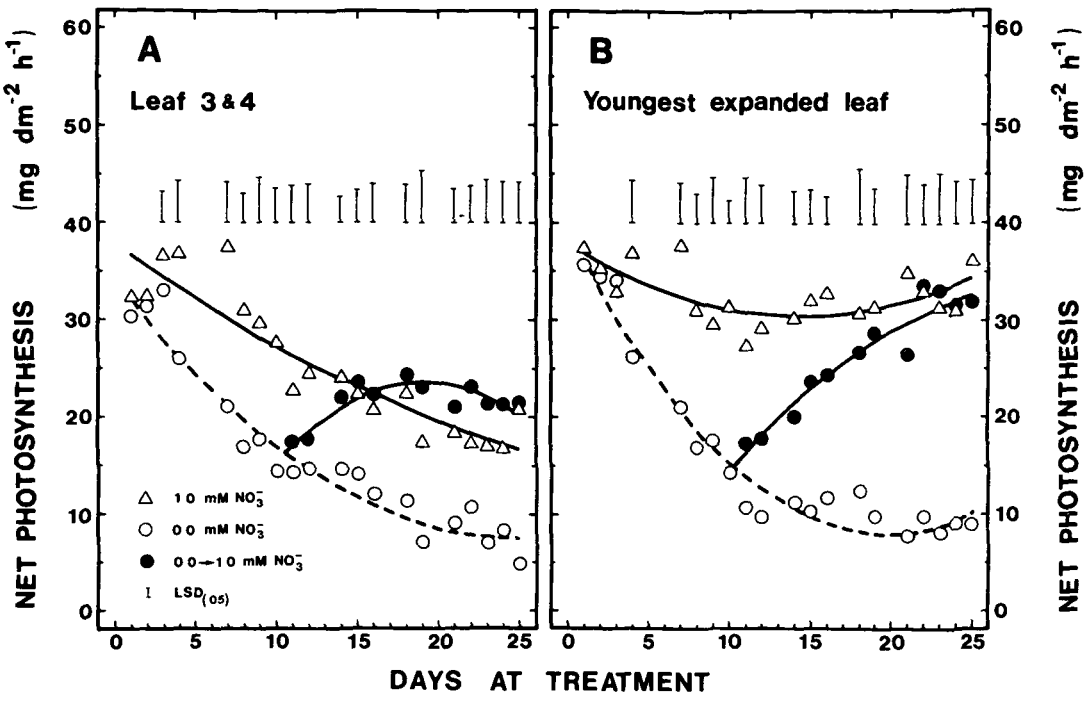


Figure 4. Effect of nitrogen stress and restoration of nitrogen availability on photosynthetic rate of (A) older, mature leaves, and (B) youngest fully-expanded leaves on the day of measurement.

of the canopy was reduced significantly, the availability of photosynthate in the leaf pool for translocation to stems and roots was increased.

FLUCTUATIONS IN NITROGEN UPTAKE RATE

Another inference from the model (Figure 1) is that uptake rates of nitrogen, rather than remaining constant during growth, should oscillate with a periodicity related to the interval of leaf emergence and the associated changes in sink activity of leaf growth. To confirm this inference of the model, soybean plants were grown in the hydroponic system for 31 days with

a complete nutrient soluction containing 1.0 mM NO_3^-. The depletion of NO_3^- from solution was monitored daily by ion chromatography, recorded, and NO_3^- was added to the solution as $Ca(NO_3)_2$ to return the concentration in solution to 1.0 mM NO_3^-. Solutions were completely changed every 2 days to avoid depletion of any nutrient below 80% of the initial concentration. Uptake rate of NO_3^- per plant during each 24-h period was calculated as mM NO_3^- removed from the solution in each hydroponic system divided by number of plants in the system during that day.

As predicted by the model (Figure 1), uptake rate of NO_3^- oscillated between maxima and minima with a periodicity of 3 to 5 days (Figure 5A). The interval between emergence of successive mainstem soybean leaves is

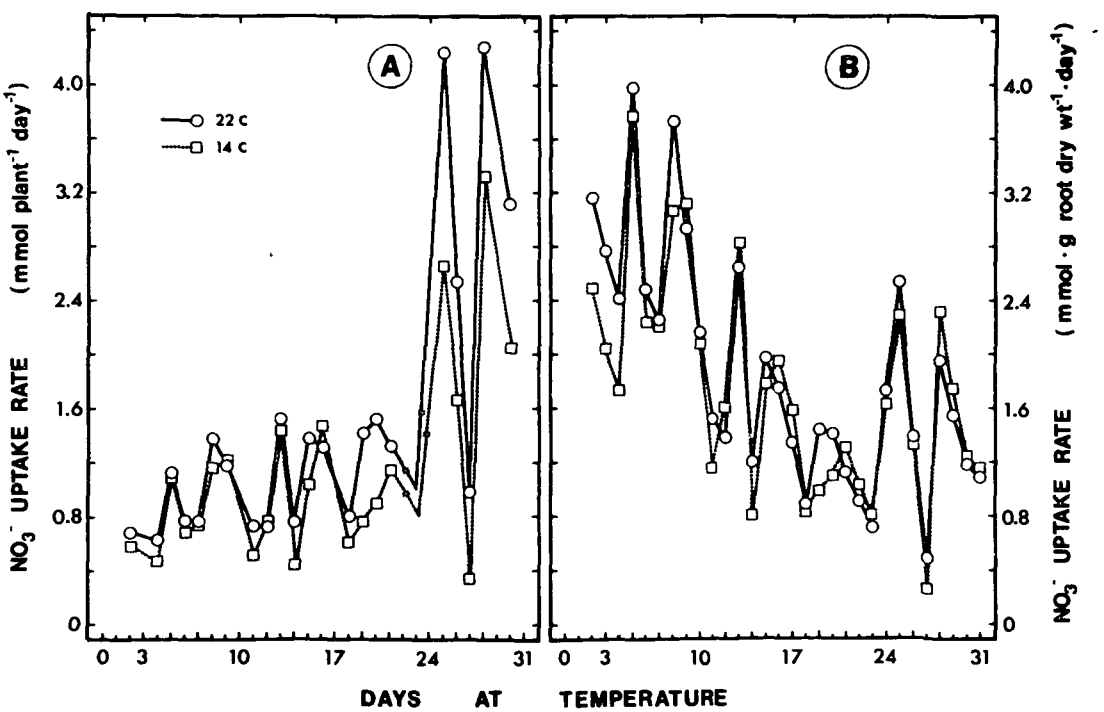


Figure 5. Uptake rate of NO_3^- per plant (A) and per g root dry weight (B) of soybean plants grown at two root-zone temperatures. Uptake rates were determined by depletion of NO_3^- from a replenished nutrient solution. (Adapted from Tolley and Raper, 1985.)

about 4 days at the 26/22 C day/night aerial temperature used in this study (Hesketh et al., 1973; Thomas and Raper, 1976). The periodicity of the oscillations in NO_3^- was not affected by the root-zone temperatures of 14 or 22 C, although uptake rates per plant were lower at 14 then 22 C. The study was repeated with concentrations of nitrogen in solution at 10.0 and 1.0 mM NO_3^-. Although the minima and maxima of the osicllations were greater at 10.0 than 1.0 mM NO_3^-, the periodicity of oscillations remained between 3 and 5 days (data not shown). These results indicate that, while the maximum rate of uptake may be regulated at the root level, the control of the actual uptake does not reside in the roots themselves, but is a function of the interdependence of roots and shoots.

DISTURBANCE OF ROOT FUNCTION

When root function is disturbed, the model indicates that an alteration should occur in rate of nitrogen uptake as the plant establishes a new balance between root and shoot function. The previous experiment serves to test this hypothesis since plants were grown at root temperatures of 14 and 22 C while aerial conditions remained the same. Total plant and root dry weights were reduced slightly at the 14 C, relative to the 22 C, root temperature (Figure 6). Nitrogen accumulation in the plants at 14 C was reduced proportionally with the dry weight (data not shown).

During the initial 5 to 10 days following transfer of the plants to the 14 C root temperature, the uptake rate of NO_3^- per g root dry weight was lower at 14 than 22 C (Figure 5B). After this initial period, there was little

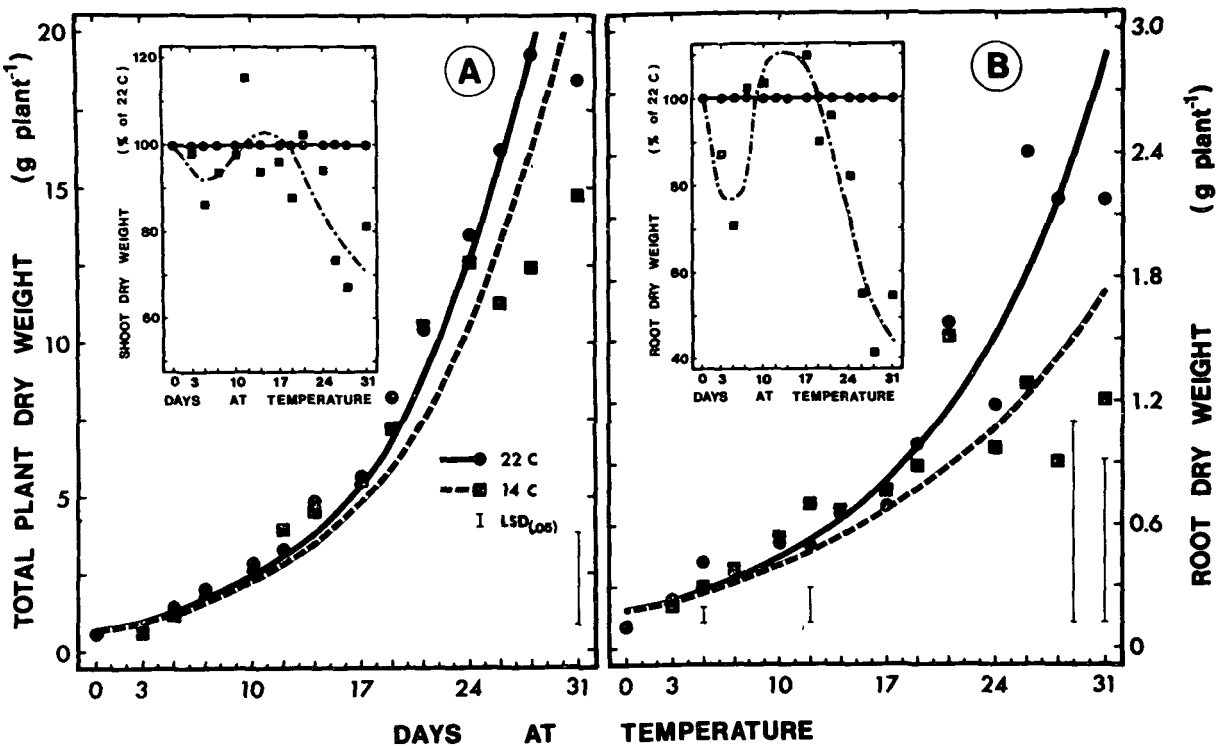


Figure 6. Effect of root-zone temperature on dry matter accumulation in (A) whole plants, and (B) roots of soybean. Shoot (A) and root (B) dry weights at 14 C are shown in insets as percentages dry weights at 22C. (Adapted from Tolley and Raper, 1985.)

difference in uptake rate per g root. The initial reduction in rate of NO_3^- uptake per g root presumably was a direct response of root metabolism or membrane permeability to the lower temperature (Osmond et al., 1982). As root growth continued, however, uptake rate per g root at 14 C became indistinguishable from that at 22 C. Thus, the initial reduction in uptake rate of NO_3^- per plant (Figure 5A), as well as the initial decrease in total nitrogen accumulation by plants (data not shown), at 14 C can be attributed to an effect of temperature on the absorption processes of NO_3^- by roots.

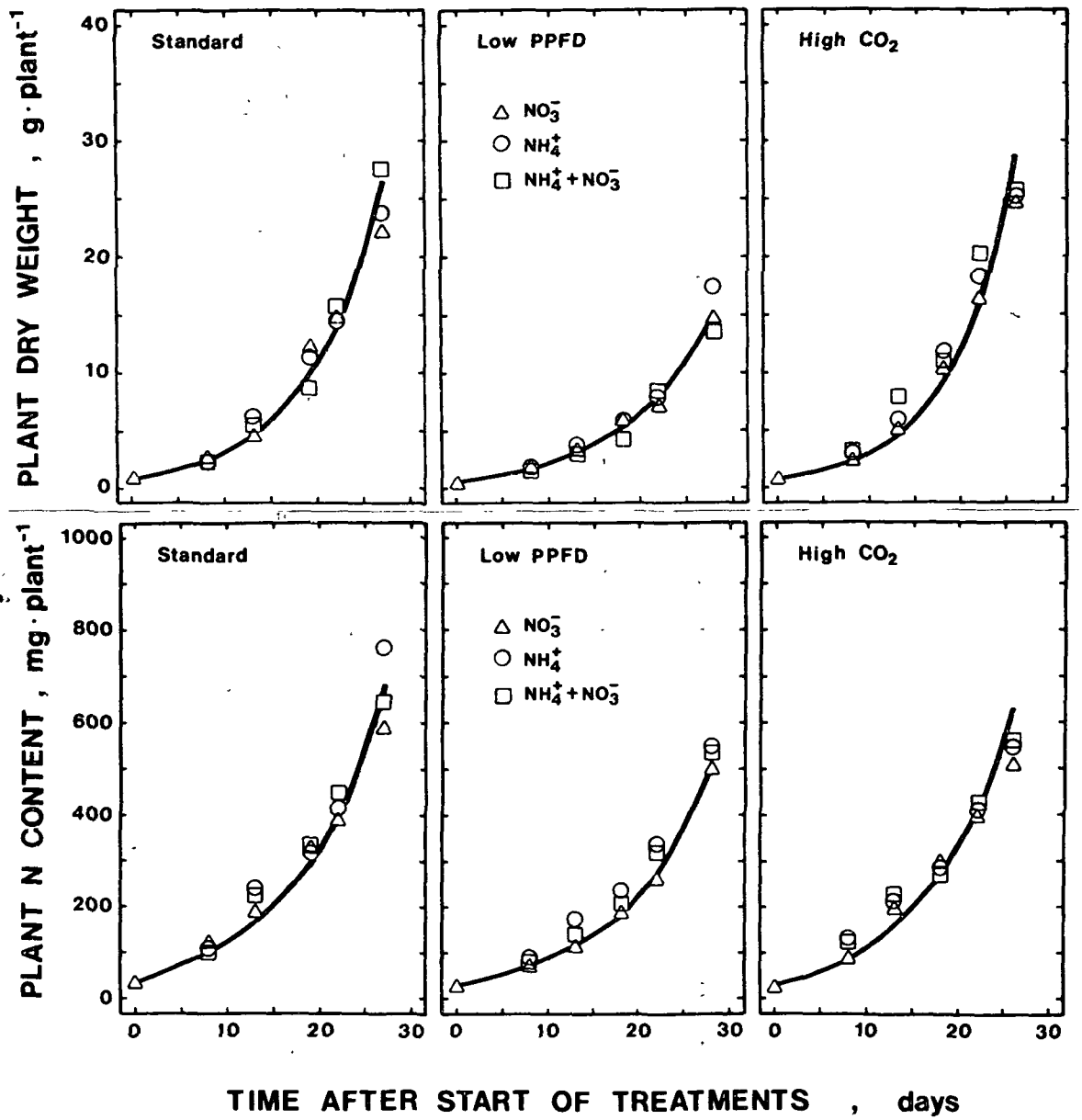
The continued reduction in uptake rate of NO_3^- per plant at 14 C after the initial period of exposure is a consequence of the reduction in root growth (Figure 6B).

UTILIZATION OF AMMONIUM AND NITRATE SOURCES

Plants supplied with moderate concentrations of NH_4^+ in solution generally grow poorly compared with plants supplied with NO_3^-. Experiments were conducted in the flowing hydroponics system to determine whether growth restrictions could be avoided over an extended period in the presence of NH_4^+ if root-zone pH were controlled and if plants were exposed to NH_4^+ during exponential growth when carbohydrate fluxes to the root are coordinated with the rate of nitrogen acquisition.

In an initial experiment, vegetative soybean plants at the beginning of the exponential growth stage were transferred to complete nutrient solutions containing nitrogen as either 1.0 mM NO_3^- or 1.0 mM NH_4^+. Acidity of the solutions was constantly monitored and maintained at pH 5.8. Experiments were conducted under three sets of aerial environments: (1) standard conditions with PPFD of 700 μmol $s^{-1}m^{-2}$ and CO_2 at 400 μL L^{-1}, (2) low PPFD conditions with PPFD of 350 μmol $s^{-1}m^{-2}$ and CO_2 at 400 μL L^{-1}, and (3) high CO_2 conditions with PPFD of 700 μmol $s^{-1}m^{-2}$ and CO_2 at 1000 μL L^{-1}. The source of nitrogen did not alter growth or nitrogen accumulation of plants over a 4-week growth interval (Figure 7) under any of the three environmental conditions. In a related experiment with tomato (Lycopersicon esculentum L. Mill. 'Vendor'), growth and nitrogen accumulation by plants during exponential

Figure 7. Effect of NO_3^- and NH_4^+ sources on dry matter and nitrogen accumulation in soybean plants grown under standard (PPFD = 700 µmol $s^{-1}m^{-2}$ and CO_2 = 400 µL L^{-1}), low PPFD (PPFD = 325 µmol $s^{-1}m^{-2}$ and CO_2 = 400 µL L^{-1}), and high CO_2 (PPFD = 700 µmol $s^{-1}m^{-2}$ and CO_2 = 1000 µL L^{-1}) conditions. (Adapted from Rufty et al., 1983.)



growth were not altered by source of nitrogen when acidity of the solution was controlled at pH 6.0 (data not shown).

In a subsequent experiment, soybean plants were grown with 1.0 mM NH_4^+ as the sole nitrogen source with acidity of the solution maintained at pH 6.1, 5.1, and 4.1. While plants exposed to NH_4^+ at pH 6.1 accumulated dry matter (Figure 8) and nitrogen (data not shown) at rates comparable to plants exposed to NO_3^- as the sole nitrogen source (cf. Figure 7), growth was reduced at pH 5.1 and ceased within days of initial exposure to NH_4^+ at pH 4.1. The decreased growth at low pH under NH_4^+ nutrition was not a singular response to acidity of the nutrient solution. In another experiment (Rufty et al., 1982) soybean plants receiving 1.0 mM NO_3^- as the nitrogen source initially responded to decreased solution acidity from pH 6.1 to pH 4.1 with a reduction in growth rate, but the plants receiving NO_3^- acclimated to the low pH and after 3 weeks had attained growth rates comparable to plants growing at pH 6.1. Apparently, there is an interaction between external pH of the nutrient solution and the ability of the plants to assimilate NH_4^+ as it enters the roots. Experiments are planned to further explore the relationship between external acidity and NH_4^+ toxicity.

CONCLUSION

The results of our experiments support the proposed conceptual model (Figure 1) that relates nitrogen uptake activity by plants as a balanced interdependence between the carbon-supplying function of the shoot and the nitrogen-supplying function of the roots. The data are being used to modify

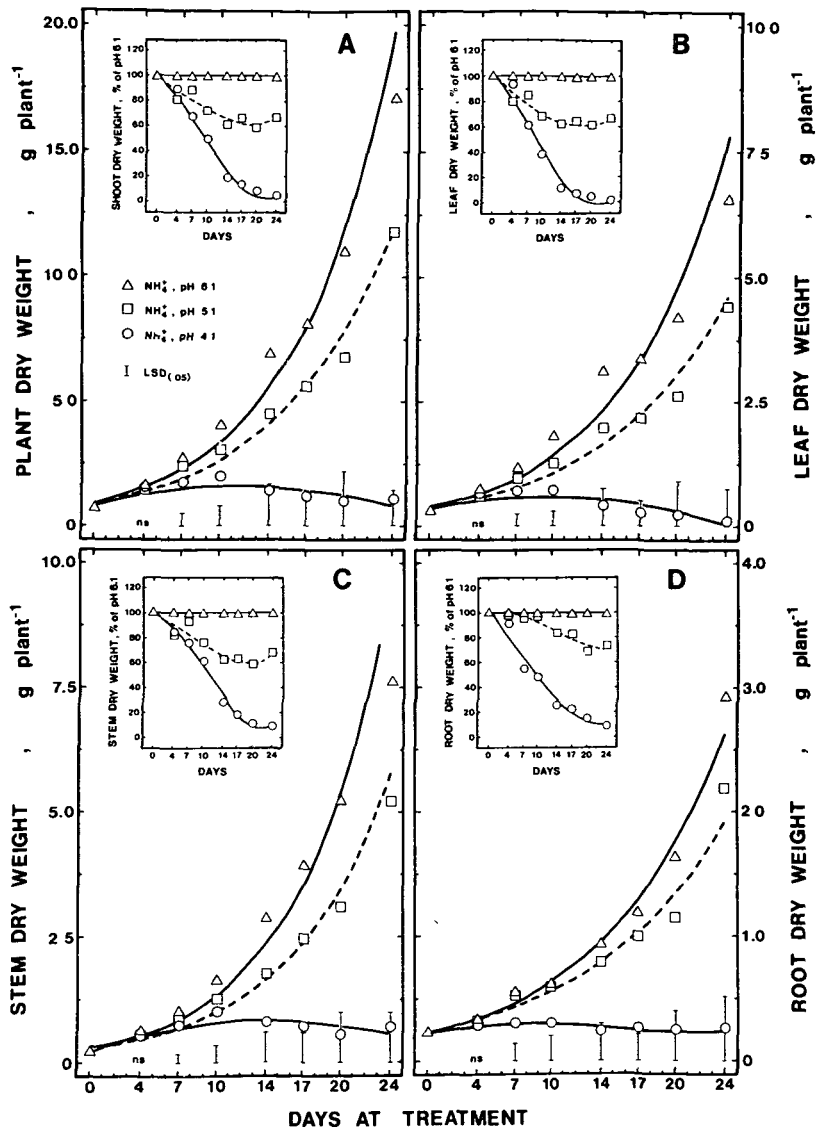


Figure 8. Effect of pH of the nutrient solution on dry matter accumulation by soybean plants receiving NH_4^+ as the sole nitrogen source.

a dynamic simulation model of plant growth, which presently describes carbon flows through the plant (Wann and Raper, 1979, 1984), to describe nitrogen uptake and assimilation within the plant system. Although several models have been proposed to predict nitrogen uptake and partitioning, they emphasize root characteristics affecting nutrient uptake and rely on empirical methods

to describe the relationship between nitrogen and carbon flows within the plant. We propose, on the other hand, to continue to attempt a mechanistic solution in which the effects of environment on nitrogen (as well as carbon) assimilation are incorporated through their direct effects on photosynthesis, respiration, and aging processes.

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THE ROLE OF PLANT DISEASE IN THE DEVELOPMENT OF CONTROLLED ECOLOGICAL LIFE SUPPORT SYSTEMS.

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INTRODUCTION

Controlled Ecological Life Support Systems (CELSS) for human habitation are proposed for long duration exploration of extraterrestrial habitats. The prime candidates for the photoautotropic components of CELSS are edible higher plants. These plants would provide food and oxygen and perform other functions needed for human life. A primary concern in the design and operation of a CELSS based on higher plants is maintaining plant health. Without continuous growth of healthy plants a CELSS would not function properly. Although many aspects of plant growth have been addressed by scientists in the CELSS program, a subject that has received minor consideration is plant disease. Plant disease can be defined as any disturbance of a plant that interferes with its normal structure, function or economic value (2).

IMPORTANCE OF PLANT DISEASE

Higher plants are the most important sources of human food, but their production requires a constant and intensive effort to control diseases, both in the field and in semi-controlled

environments such as in greenhouses (2). Crop loss due to disease is substantial (5). The U.S.D.A. estimated that $3.7 billion were lost to crop diseases in the United States in 1965 (1). However, the annual overall impact of disease is much greater since an incalculable cost is involved in controlling disease to prevent preharvest and postharvest crop loss. Just one plant disease can have a major impact on crop production. The 1970-71 epidemic of southern corn leaf blight in the United States caused an estimated $1 billion loss (22). The role of plant disease in the production of healthy plants can not be overemphasized.

There are over a thousand microorganisms that cause plant diseases (2). They represent diverse biological groups such as viroids, viruses, mycoplasmas, spiroplasmas, bacteria, fungi and nematodes. Because of this diversity, there are a wide variety of ways in which pathogens survive, reproduce, disseminate, and infect plants and cause disease. Many pathogens also are highly variable genetically. These factors are some of the reasons why plant diseases are common, destructive and difficult to control (2). Furthermore the genetic uniformity in our important food and fiber crops has contributed greatly to plant disease epidemics (14).

WILL PLANT DISEASE OCCUR IN CELSS.

The important question concerning plant health in CELSS is: will plant diseases be a problem? There is good evidence to suggest that they will indeed occur. At the present time it appears that quarantine is considered the principal means of preventing the introduction of plant pathogens into CELSS. Quarantine programs, however, are not always completely successful (18). The introduction of coffee rust into Central America and downey mildew of maize and sorghum into the United States are just two examples of important diseases not controlled through quarantine.

Because of the diversity of plant pathogenic microorganisms, for example, in size, and the numerous means of dissemination, controlling their movement is notoriously difficult. Consider the numerous organisms that are seed borne either within or on seed (true seed or vegetative cuttings) (15). These organisms can be difficult to detect and are often the primary inoculum for disease (2). Potato production, for example, requires a constant indexing of seed tubers for presence of viruses (8). Plant pathogens also have a wide variety of survival mechanisms (2). Many can tolerate extremes of temperature and moisture and survive for many years in the absence of a host. Some are latent in their host and cause disease only under specific conditions. Many fungi pro-

duce airborne propagules, often in abundance, which are readily disseminated and can easily contaminate equipment and humans. There are also numerous pathogens that are disseminated and introduced into plants by insect vectors such as aphids and mites.

A variety of fungi and bacteria have been detected aboard spacecraft, either on the hardware or the human passengers. In the Apollo 16 and Skylab spacecrafts, 16 fungal genera that contain plant pathogens were detected (4,7). Some of these genera such as Fusarium, Helminthosporium, Alternaria, Phoma and Cephalosporium contain very destructive plant pathogens (2). The potential for introducing specific plant pathogens into CELSS was demonstrated in a Skylab simulation test where 8 species of plant pathogenic fungi were detected on the human participants (6). Human carried bacteria which cause disease on both plants and animals also may be introduced into CELSS (13).

Fungi, bacteria and viruses survive direct exposure to the space environment even for months if shielded from direct solar radiation (9,11,20,21,23). Tobacco mosaic virus and Bacillus subtilus are examples of two plant pathogens which remained viable after exposure to the space environment (9,21). Indeed, the space environment of deep cold and vacuum

are used by plant pathologists in the lyophilization process to preserve plant pathogens. This indicates that contamination of spacecraft and cargo outside of the life support system could introduce plant pathogens into a space station utilizing a CELSS. In view of the preceding evidence, the concept that quarantine procedures could completely exclude plant pathogens from CELSS is unrealistic.

Another important point to consider is the vulnerability of CELSS to plant disease. If plant growth symptoms such as aeroponics, misting, thin films, etc., lack a natural microbiological community, there will be no natural competition to prevent establishment and spread of pathogens. This can result in rapid and destructive disease development. This phenomenon is well known in plant pathology (3,10). The recolonization of steam sterilized greenhouse beds by Fusarium oxysporum and the development of tomato wilt is a good example of serious disease resulting from the lack of a biological buffer (16).

The plant growth systems proposed for CELSS (12) also may be vulnerable to disease induced by non-infectious microorganisms (exopathogens). These microorganisms could adversely affect plant growth through competition for rooting substrate and/or liberation of toxins that inhibit normal plant growth (26).

These diseases could be severe in a system lacking a normal rhizosphere flora.

Eight higher plant species have been recommended for prime consideration in CELSS: wheat, rice, potato, sweet potato, soybean, peanut, lettuce, and sugarbeet (25). Of those, wheat, potato and soybean were recommended for intensive research to obtain baseline information for evaluating the use of plants in CELSS (25). All of these crops however are susceptible to a wide variety of plant pathogenic microorganisms (8,17,24). For example, at least 200 diseases have been described on wheat but about 50 are routinely important economically (24).

EFFECTS OF PLANT DISEASES

There are four important effects that plant disease could have in CELSS. These are: (a) complete destruction of plants, (b) reduction in efficiency of plant growth, (c) destruction of the useful parts of plants and (d) creating sanitation and environmental contamination problems.

Damping off and root diseases (i.e. soil borne diseases) are those most likely to result in complete destruction of plants. The plant growth systems proposed for CELSS would be highly vulnerable to those diseases since many root pathogens require

high water potential for activity (2) and normal rhizosphere flora would be absent. Also, water delivery systems to roots could become contaminated and act as a means for rapid dissemination of pathogens.

The efficiency of plant growth could be affected by numerous pathogens which attack only certain tissues such as feeder roots, leaves or stems. Powdery mildew is an example of a disease where the pathogen usually does not kill the plant, but can severely reduce growth (19). Powdery mildews are often a serious problem in semi-controlled environments such as greenhouses.

Infection of edible or usable parts of higher plants by pathogens can destroy, reduce the quality or adversely affect processing or storage of the plant product. The cereal smuts, for example, completely replace the grain with smut spores. The production of mycotoxins in fruits, vegetables and seeds is an example of a reduction in quality and a health hazard. Aspergillus flavus one of the fungi found aboard spacecraft, (4) is a principal producer of a mycotoxin found in infected cereals and legume seeds (2).

Plant diseases would also necessitate the implementation of sanitation procedures, requiring time and energy, to destroy

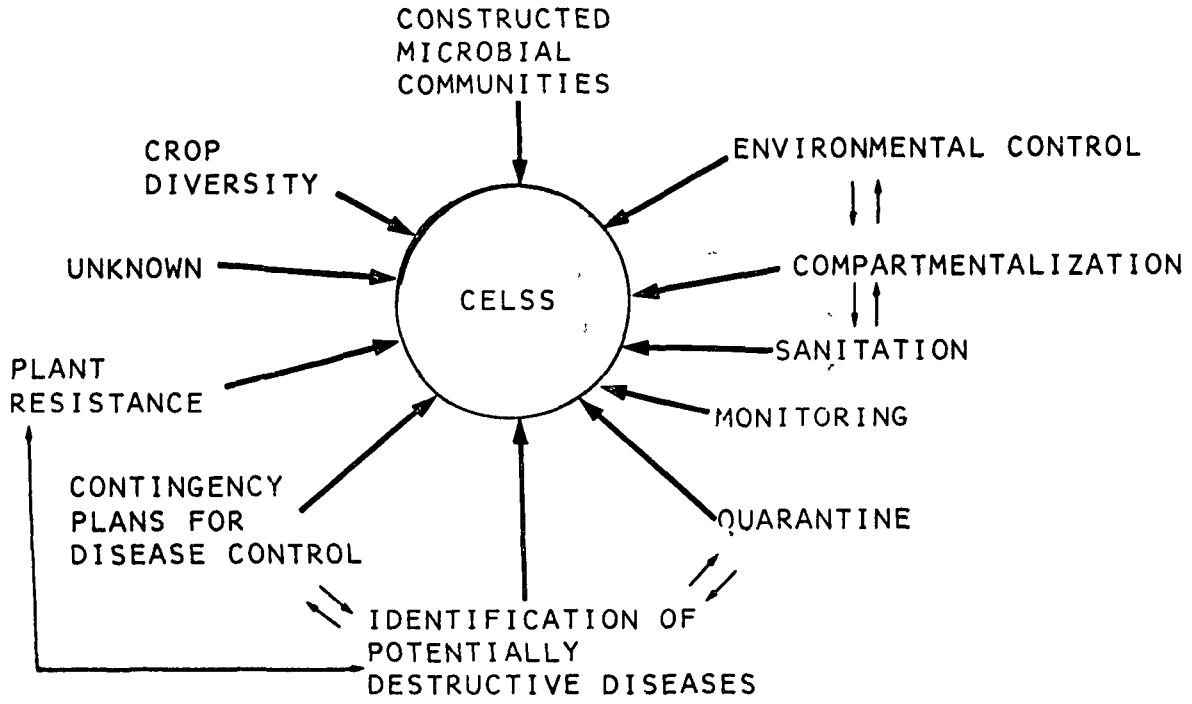

FIG. 1. SCHEMATIC DIAGRAM OF AN INTEGRATED PLANT DISEASE CONTROL PROGRAM.

disease inoculum (2). The greater the complexity of a growth system the more difficult the procedure to successfully eliminate inoculum. Plant debris, the physical parts of the rooting area, the rooting medium and the water delivery system would all need sterilization.

There is a potential problem of environmental contamination associated with some diseases. Plant pathogenic fungi can produce large quantities of spores on plants and these spores are liberated into the air and might be a health hazard in a closed system. Also, the control of certain diseases can be achieved with chemicals. However, air and water contamination could occur as a consequence of chemical applications. In CELSS, chemical control may be undesirable.

PLANT DISEASE CONTROL IN CELSS

Plant diseases in CELSS can be prevented or their damage minimized with an integrated disease control program. The important components (and their interactions) of such a program are diagrammed in Figure 1. They are as follows:

1. Quarantine. An important step to prevent introduction of pathogens. NASA should establish a policy that only pathogen free plants (seed and vegetative stock) are introduced into CELSS. The policy should be implemented prior to the development of an operational CELSS for a permanent

space station.

2. Identification of diseases most likely to occur in CELSS. Such information can be gained from current knowledge on plant disease, microbial contamination of spacecraft and astronauts, and from land based CELSS experiments. This information is fundamental to all aspects of integrated disease control.
3. Plant resistance. Host resistance should be utilized for those diseases which are potentially most destructive in the CELSS environment.
4. Crop diversity. When feasible, diverse crops should be utilized to minimize the impact of damage from plant disease.
5. Constructed microbial communities. The utilization of constructed beneficial rhizosphere and phylloplane microbial communities would act as a buffer against increases in pathogen populations and reduce infections. This would be true biological control. Such microbial communities might also prevent problems with exopathogens and could directly benefit plant growth (for example, through mycorrhiza). Maguire (13) has addressed this concept in a NASA contracted report.
6. Environmental control. Controlling environmental factors such as temperature and humidity are important methods of preventing or minimizing disease development.

7. Compartmentalization. Dissemination of pathogens and loss from disease can be minimized by compartmentalization of plant growth systems. This should include water delivery systems, plant handling equipment and processing areas. This would greatly facilitate sanitation procedures.
8. Sanitation. The destruction or removal of inoculum is often essential to prevent further disease occurance. Since plant growth systems will most likely be in constant use, sanitation will be an extremely important disease control procedure. Plant growth systems should be designed for fast and efficient sanitation (i.e. sterilization or pasteurization). In semi-controlled environments such as greenhouses, sanitation is widely practiced for disease control.
9. Monitoring. Disease monitoring is necessary to implement specific disease controls, prevent spread of pathogens and provide information upon which future disease control decisions are based. Due to the greater value of plants in CELSS, disease monitoring will be more important than in earth agriculture. Sophisticated disease monitoring systems such as measuring reflected radiation in specific infrared wavelengths could be utilized in CELSS.
10. Contingency plans to control epidemics. Control procedures should be formulated for plant disease epidemics

that might occur in the CELSS environment. For example, powdery mildew may be a potential threat in controlled environments. Although powdery mildew can be controlled with sulfur dust, such a control may not be practical in CELSS. What specifically would be the control to prevent a powdery mildew epidemic?

CONCLUSION

Plant diseases could be important factors affecting growth of higher plants in CELSS. Disease control, therefore, will be needed to maintain healthy plants. The most important controls should be aimed at a) preventing the introduction, reproduction and spread of pathogens and b) preventing plant infection. An integrated disease control program will maximize that approach. In the design and operation of CELSS, plant disease should be considered an important aspect of plant growth.

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EPILOGUE

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Development of Space Technology for Ecological Habitats

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Introduction

NASA's announcement that it will develop a Space Station over the next decade initiates a new phase of human evolution into space. When the Space Station becomes operational, it will permit continuous human presence in the space environment well into the next century. Scientific experiments that require long periods of time in the space environment will then become possible. Among the many aerospace technologies that make the long term presence of humans in space possible is the technology of life support.

Because of current life support technology, astronauts can work in shirt sleeves inside of pressurized spacecraft, and survive in sealed suits surrounded by the vacuum of space. Life support on spaceflights conducted by the U.S. and the U.S.S.R. over the past 25 years was possible because enough air, water and food was carried from Earth to maintain life. Longer missions have involved replacing supplies of oxygen and food from Earth, in the case of the Soviet cosmonauts on the Salyut orbital station, or electrolyzing water to produce oxygen, in the case of the U.S. Skylab mission. For the most part, however, the regeneration of life support supplies has had limited use.

As flight duration and crew size increase, resupply of life support materials from Earth becomes noticeably expensive. Recycling of spent life support supplies reduces payload weight and the cost of resupply from Earth. On Salyut for example, water vapor from human respiration and perspiration was condensed from the cabin atmosphere on cooling coils. This condensate, along with used wash water was passed through ion exchange columns and activated charcoal filters, sterilized by heat, and stored.

Both the U.S. and the U.S.S.R. have expressed interest in, and actively support research in a variety of techniques to regenerate life support materials in space. The American program has explored two approaches to the problem. One is based on physical-chemical techniques that show promise in regenerating oxygen and clean water; the other makes use of those mechanical technologies with aspects of biotechnology to integrate photosynthetic organisms into the system. The American program is called Controlled Ecological Life Support System (CELSS) research. There is considerable evidence from the open literature that the U.S.S.R. space program is following similar lines of research. Material cycling in a CELSS (Figure 1) could sustain life in space for an indefinite period, while reducing the cost of human space operations.

The technology of CELSS includes physical-chemical-mechanical systems that use heat, pressure and chemical reactants to process food, oxidize wastes, separate and store gases, as well as biological systems that use either micro-algae or angiosperms (higher plants) to produce food, potable water and oxygen, and to remove carbon dioxide. CELSS may be an enabling technology for long-term missions on the Moon and to Mars and the outer planets. A sample scenario is first to develop CELSS technology for use on later Space Stations, then

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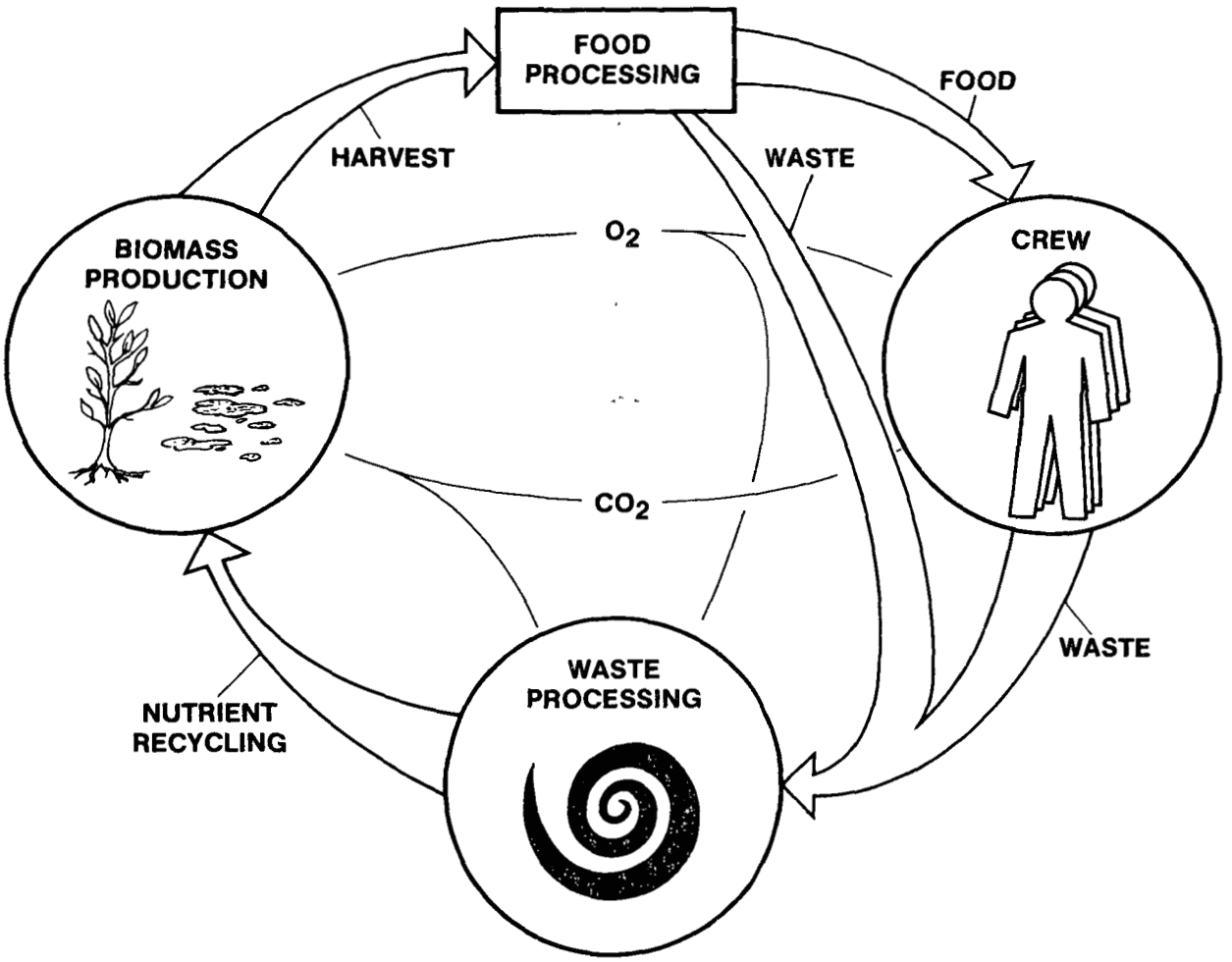

Figure 1. Pathways of material cycling within a CELSS

using CELSS technology on the lunar surface, or to establish a habitat on the Martian moon Phobos. The experience gained with habitats on these moons would be essential for the establishment of a habitat on the Martian surface, which has its own unique environmental conditions and design problems for habitation. Space Station experience would also be invaluable in developing scenarios using CELSS technology for transit to Mars or elsewhere in the solar system.

Biological Space Research Relevant to CELSS

Two approaches to developing a CELSS have been suggested. A holistic method involves enclosing an ecosystem, altering the species content, and manipulating environmental conditions until long-term stability and productivity is achieved. Another approach is a reductionist method that divides the system into several subsystems, develops separate controls for the complete system as well as for each subsystem, and links the subsystems and their controls together. Generally speaking, researchers in the U.S.S.R. have chosen the holistic strategy, while those in the U.S. have chosen the latter. Although in some respects their approaches are different, both the Soviet and American space programs have made considerable advances in bioregenerative life support research.

One of the central research tools of bioregenerative research is the tightly-sealed plant growth chamber. This kind of device, several of which have been built in France (see papers by M. André, these proceedings), and in the U.S. (see paper by Schwartzkopf, these proceedings), provide an opportunity to examine the metabolism of plants while they are growing and to experimentally manipulate environmental conditions to increase (or decrease) growth rates, oxygen production, etc. Typically, a chamber contains separate compartments for the roots and shoots. The stem compartment allows the leaves to grow in higher than normal levels carbon dioxide, lower oxygen tensions, or both to promote photosynthesis and plant growth, to stimulate flowering, and to produce higher crop yields. A separate compartment for roots growing hydroponically or aeroponically (misting the roots) allows manipulation of the rhizosphere (root zone), as well as studies of nutrient uptake. The Schwartzkopf chamber is diagrammed in Figure 2.

Only recently with the advent of computer control systems has it been possible to achieve in enclosed chambers the necessary manipulation of air movement, water, humidity, and temperature and the removal of contaminants to maintain long-term plant growth. Although these chambers are a specialized requirement for research and development of a CELSS, they can also have terrestrial applications. This new technology expands the resources available for research by the botanist and agronomist. In addition they can be used for the study of ecosystem effects on new organisms produced by recombinant DNA techniques, or on chemical products (pesticides, herbicides, etc.) before releasing them into the environment.

The missions of the Soviet Salyut space station provided for the first time an opportunity for many long-term experiments using plants in space. Starting in the 1970's, Soviet researchers experimented with both partially and totally controlled environments for plants in space using a series of chambers, which were gradually upgraded to suit the unique environmental conditions of microgravity. Problems of water and nutrient delivery, and aeration of

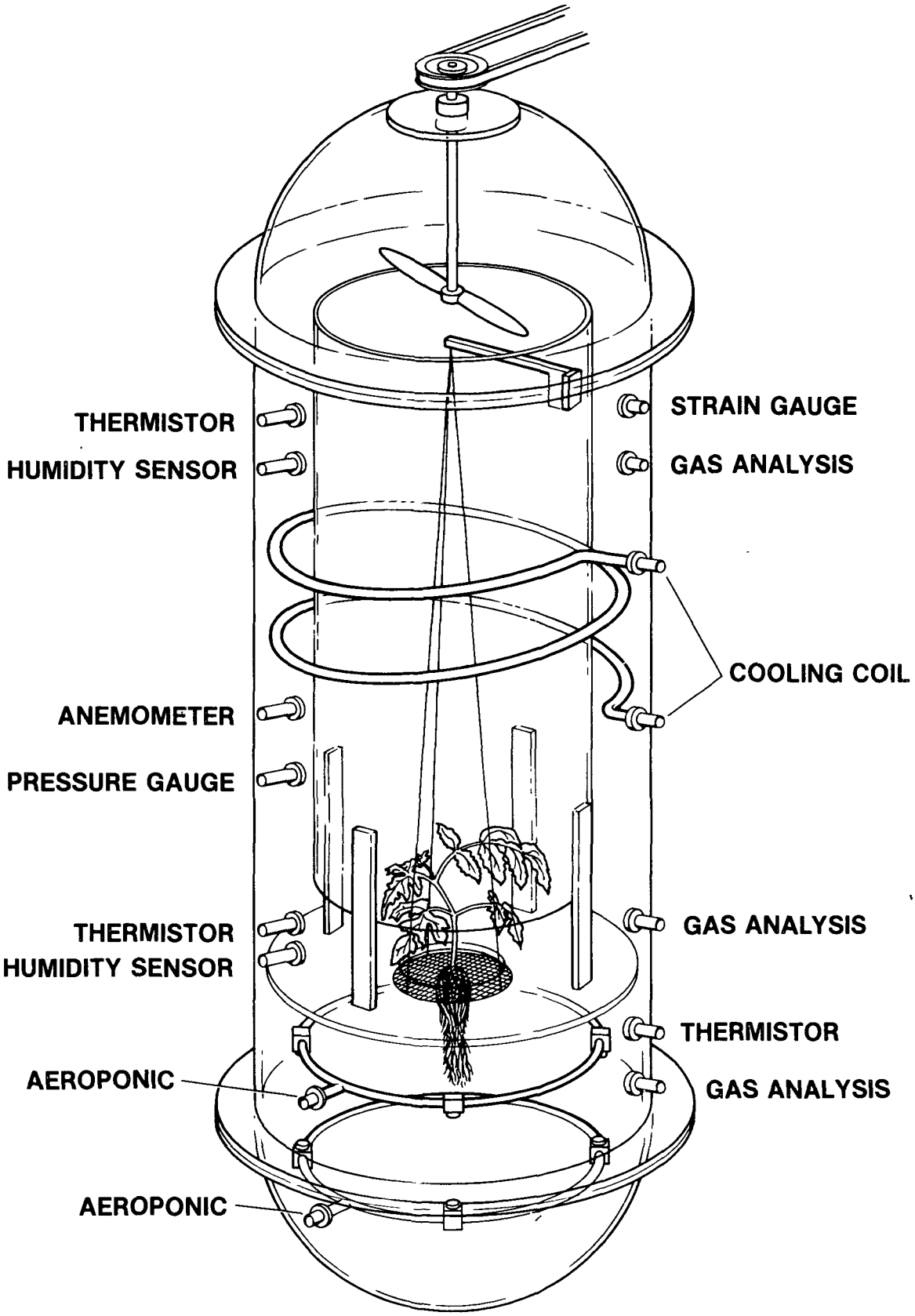

Figure 2. Closed plant chamber design that supported plant growth continuously for two months (Schwartzkopf 1985).

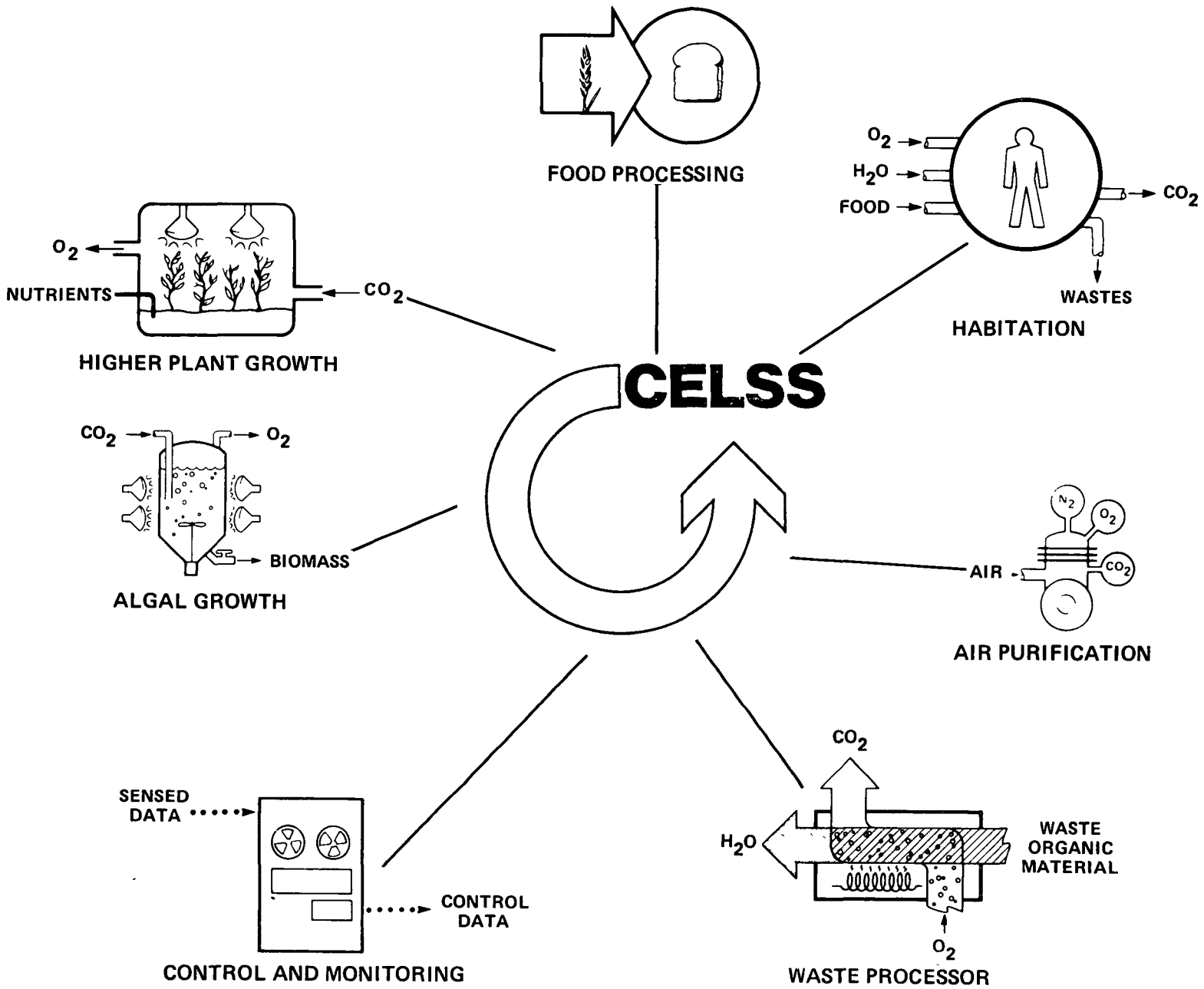

Figure 3. The various subsystems that comprise a CELSS are a mix of living and non-living components.

roots in weightlessness were apparently solved. On Salyut the growth of onion, radish, dill, cucumber, and carrot was achieved, but the plants died at the flowering stage. In 1982 flowering and seed formation in space was achieved through the efforts of Lithuanian botanists. Intense illumination and filtering impurities from the plants' air supply finally resulted in growth through an entire life cycle (fertilization, embryogenesis, maturation, and new seed formation) of the test-tube size angiosperm *Arabidopsis thaliana* (L.) Heynh. Light intensity and photoperiod apparently must be adjusted for reliable plant productivity in space, as on Earth. Some of this data is already available in the literature for input into computer data bases and models. This information is of consequence because different plant species have different lighting requirements to ensure their growth and flowering. In space, plants also developed at a slower rate and it is unknown if this effect is due to lack of convection for heat and gas exchange, or to altered plant physiology in microgravity.

Soviet researchers have conducted botanical studies on Earth continuously for 20 years in their Bios program. Using the 'Bios' series of chambers, researchers started with 12 cubic meters, which has evolved into 315 cubic meters. In recent years a team of about 20 Soviet scientists used this chamber to supply two engineers with fresh air and water, and four-fifths of their foodstuffs for five months, the length of a round trip to Mars.

Soviet experiments with food crops in space show every sign of continuing at an aggressive pace to provide cosmonauts fresh food, psychological enjoyment, and increased life support capabilities. As recently as late 1985, cosmonauts on Salyut were investigating the growth of pepper, onion, and lettuce in different nutrients in at least two different plant growth chambers. A 'Biogravistat' also provided various spin rates to affect the growth rate of seeds in space.

Stability in Controlled Ecosystems

The stability of any ecosystem demands the orderly flow of nutrients between the living and non-living components of the system. Non-living components in the Earth's biosphere include the gigantic reservoirs of clay and humus in the soil, and the water and the atmosphere that are set in motion by the light and heat of the sun, producing the planet's weather and the cycling of elements. In a CELSS the non-living components will be mechanical units to process crops into foodstuffs and to breakdown the excess waste and extract the nutrients, and chemical units to purify the air (Figure 3). On Earth as well as in space, the living members of the system are the same: plants, animals (including humans) and microorganisms. On Earth microbes perform most of the recycling of elements in the biosphere.

With the addition of plants for food and for air and water regeneration, a life support system begins to resemble a terrestrial ecosystem, although the large size of the terrestrial system permits life processes to continue even with some perturbations in the system. On the other hand, a small interruption, if not considered during the design of controls for the system, could be a weak link in CELSS stability and, depending on the cause of the perturbation, could cause the system to become unstable and "crash". Maintaining system stability, especially when a single factor may affect the operation of the whole system, is an issue. For this reason it is important to understand ecosystem behavior and to integrate ecological concepts into the design of a CELSS.

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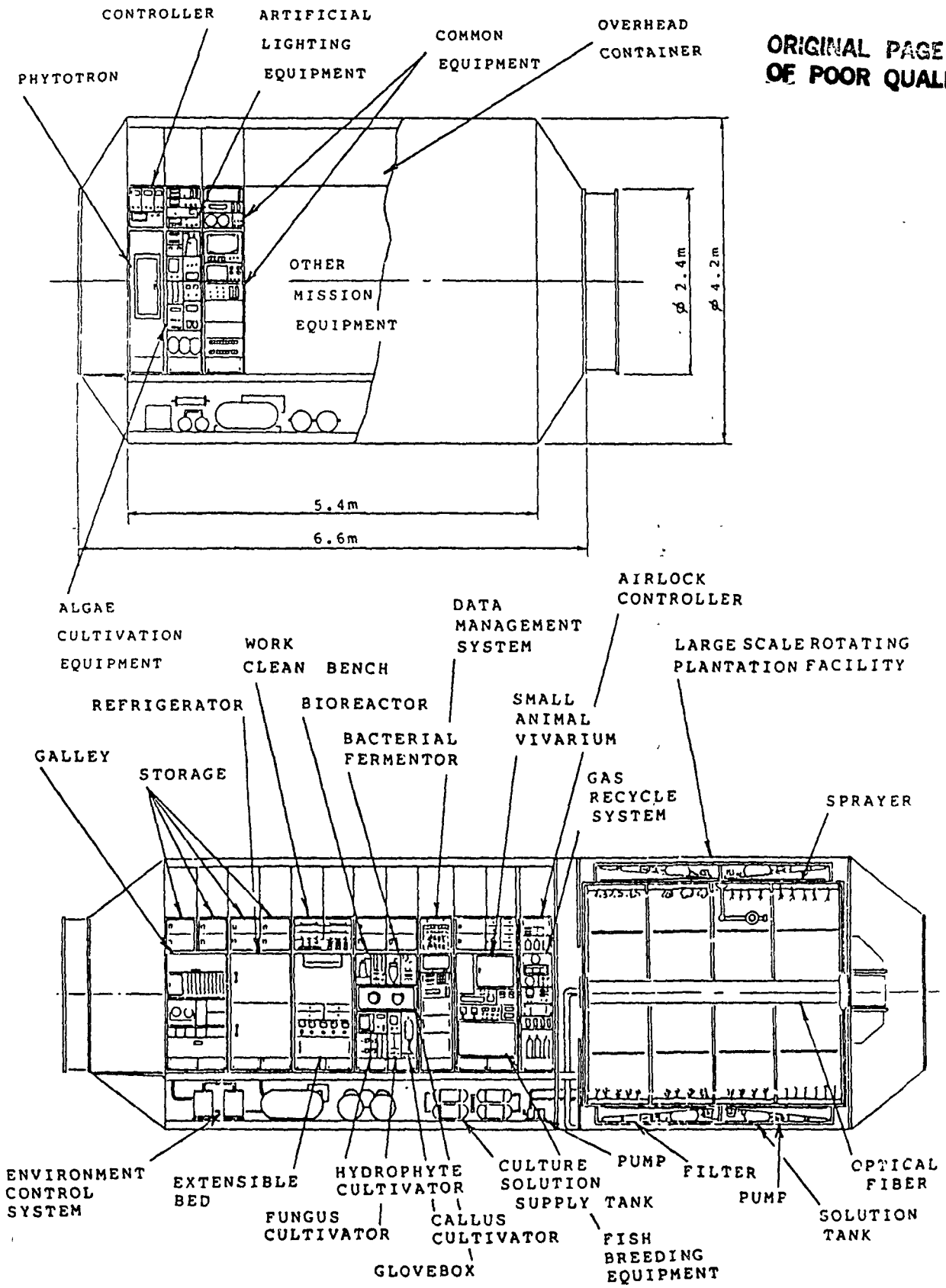

Figure 4. Japanese concept showing the evolutionary growth of a CELSS Space Station module. The first mission (top) would be based on a phytotron (plant chamber) and algal cultivator to study food production and gas conversion of carbon dioxide to oxygen through photosynthesis. A CELSS-dedicated module (bottom) eventually could be used to study a variety of organisms.

Ecological design, that is, design that incorporates principals of the natural world to sustain human settlement for a long period of time, is used successfully on Earth to maintain stability in the natural environment. One ecological design principal is the use of a variety of organisms to aid stability. For example, farms in Java have produced continuously for centuries by constant use and regeneration of water and nutrients, managed by a balanced mix of trees, livestock, grains, grasses, vegetables, and fish. No single crop type dominates the system, so if a single crop performs less than optimally, the proper flow of nutrients, and hence the whole system, will not collapse. The economy of size in a CELSS requires a limited number of specific species, carefully chosen to ensure stability and productivity. More than on crop species may even be grown in a chamber simultaneously, once studies are conducted to determine optimal species mix.

An aquaculture system has been suggested as part of a detailed design study by Japanese aerospace scientists to establish a CELSS module in space in evolutionary steps (Figure 4). To increase the complexity, and perhaps the stability, of the ecosystem 'loop' in a CELSS, bacteria could be added to transform toxic ammonia and nitrogenous waste from the fish into nitrates, which would act as a fertilizer for algae, which in turn would be a major source of food for fish. The first source of animal protein for human consumption in a bioregenerative life support system is likely to be an aquatic creature. Traditional livestock would be inappropriate in a CELSS because of their very low food conversion efficiencies. However, small animals, such as fowl and rabbits have higher efficiencies of conversion and may be of interest.

Techniques of ecological design and controlled-environment agriculture can be useful in land reclamation on Earth, as well as making permanent habitats easier to achieve in space. Recently, the University of Arizona, the New York Botanical Garden, the Smithsonian Institution and other groups proposed a seven-year venture to create an isolated, enclosed biosphere (86,000 square feet) outside of Tucson, Arizona. Such technology could be used to restore damaged ecosystems and as a research tool for studying complex ecological interactions.

Designing a CELSS for Spaceflight

Of paramount importance in a Space Station environment is the conservation of volume inside of the pressurized modules. The first CELSS for use in space must be economical, taking up as little volume as possible on the spacecraft, and recycling as much material as possible over its lifetime to minimize the launch weight of resupply from Earth. To conserve volume, the size of 'holding tanks', which act as buffers or reservoirs between the CELSS subsystems must be reduced as much as possible, and therefore material processing through the system must be rapid. Crops should produce predetermined yields in a short time. Waste materials (primarily from crop plants, but also from humans) should be quickly processed into reusable nutrients. The human control of system size and the rate of nutrient flows throughout is a major distinguishing feature of CELSS compared to the gigantic terrestrial ecosystems, their nutrient flows, reservoirs and buffers, which may require hundreds of years to completely recycle and remix elements.

If lack of normal gravity actually affects plant physiology, perhaps centrifuged chambers will be required to provide artificial gravity. If plant physiology is not the real problem in

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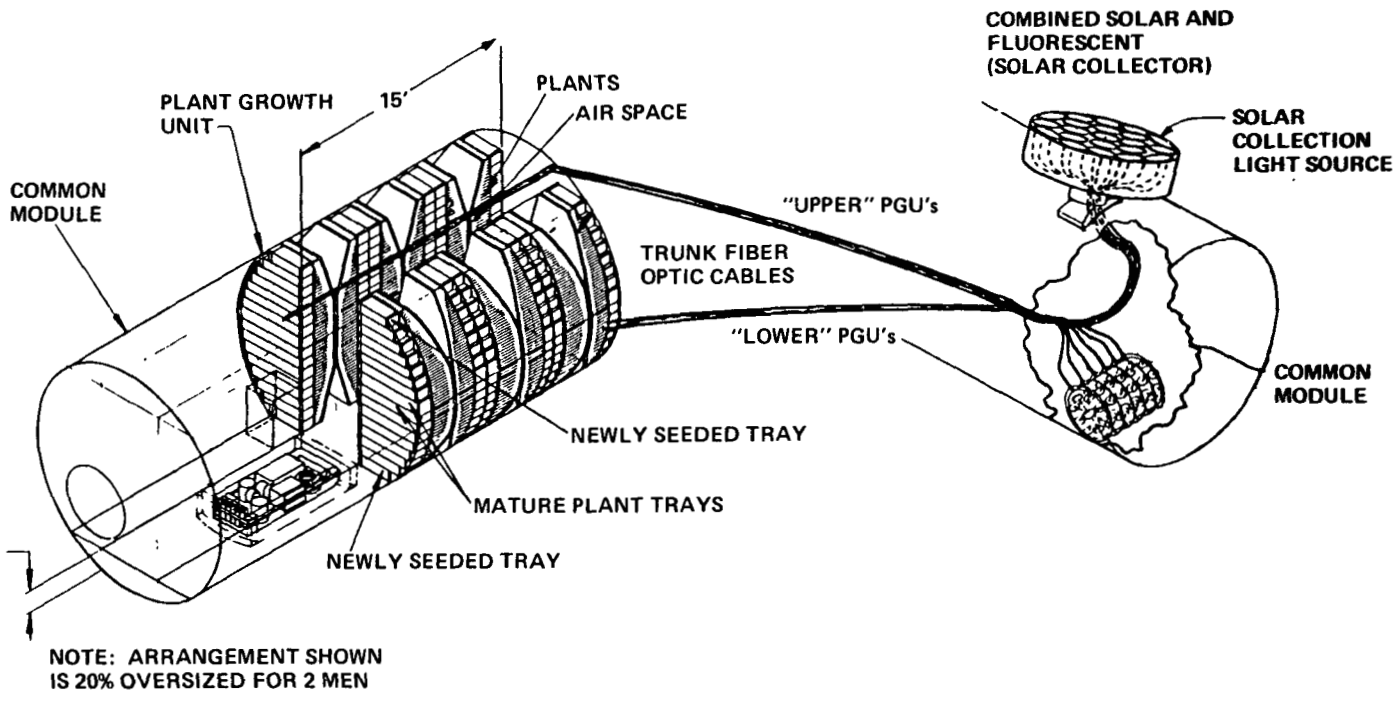

Figure 5. A combined solar and artificial lighting system delivers light to plant growth units (PGU's) via fiber optic cables.

weightlessness, more efficiently designed plant chambers will be required. In microgravity the absence of normal convection, to aid in circulation of heat and metabolic gases for the leaves, and altered behavior of fluids in the root area, may be the critical factor. The nutrient solution for the roots, easy to aerate with oxygen and drain on Earth, will be heavily influenced by the behavior of gas-liquid interfaces and the strong influence of liquids' surface tension under microgravity. On the other hand lack of response to density differences in microgravity may actually aid in the mixing of oxygen and mineral nutrients in solution. Pumps will be required just to maintain air exchange to the leaves, and water/nutrient exchange to the roots.

A particular problem in spaceflight will be the spectral quality and periodicity of light as the Space Station orbits the Earth, or the Lunar Base moves from sunlight into shadow and back. During its lifecycle a plant requires certain changes in wavelength, intensity and photoperiod to maintain normal productivity. Light must be provided in the wavelengths most usable by plants: the visible wavelengths with emphasis on red and blue, the absorption peaks of the two major chlorophyll components of plants. Existing data on the effects of light on various plant crops should be studied in this regard. New experiments should examine the unique limitations of lighting in the man-made space environment. Although maximum control can be achieved by the exclusive use of artificial lighting, this method consumes the most energy and generates the most waste heat. A mix of natural and artificial light sources may prove optimal.

To achieve energy efficiency in these space systems, natural sunlight could be collected on the outside of a CELSS module. A solar collector would remove both harmful ultraviolet light and ineffective wavelengths, such as the infrared. An extensive discussion of this approach, first proposed by a Japanese company (Himawari) has been published as a NASA report by Boeing Aerospace. A schematic design of the device is presented in Figure 5.

To ensure adequate nutrition for space crews, a minimum variety of crop species suggested for a CELSS includes soybean, peanut, wheat, rice, potato, carrot, spinach, cabbage, and lettuce. This list could be expanded to include root crops like sweet potatoes, red beets and sugar beets; vegetables like broccoli, cauliflower chard and other greens; and additional grain crops. Tomato, green bean and sugar pea may also be included. Cantaloupe and the everbearing (perennial) strawberry are suitable fruit species. To prepare the harvest for storage and consumption, automated methods must be chosen and adapted from those already in use in the food processing industries. Threshing, hulling and milling of grains and seeds; pressing, centrifugation and filtration of oils and juices; evaporation of sugars; pickling and fermentation to preserve certain foods; and dehydration of some products to minimize storage space and maximize 'shelf life' will all be required.

In a CELSS module it may be necessary to process the largest variety of foodstuffs from a few major crops that are good producers in microgravity. Some of these so-called fabricated foods are quite acceptable in the diet; some may be less so. Such foods include imitation cheeses and the traditional Oriental meat analogues of tofu, tempeh and seitan (gluten). Seasonings and flavorings for foods may best be provided from Earth by essential oils or their analogues, which are so concentrated that their payload weight may be negligible. By not growing herbs and spices in space, valuable space and energy would be conserved in a CELSS module for food crops that are essential. This strategy would also avoid a whole new class

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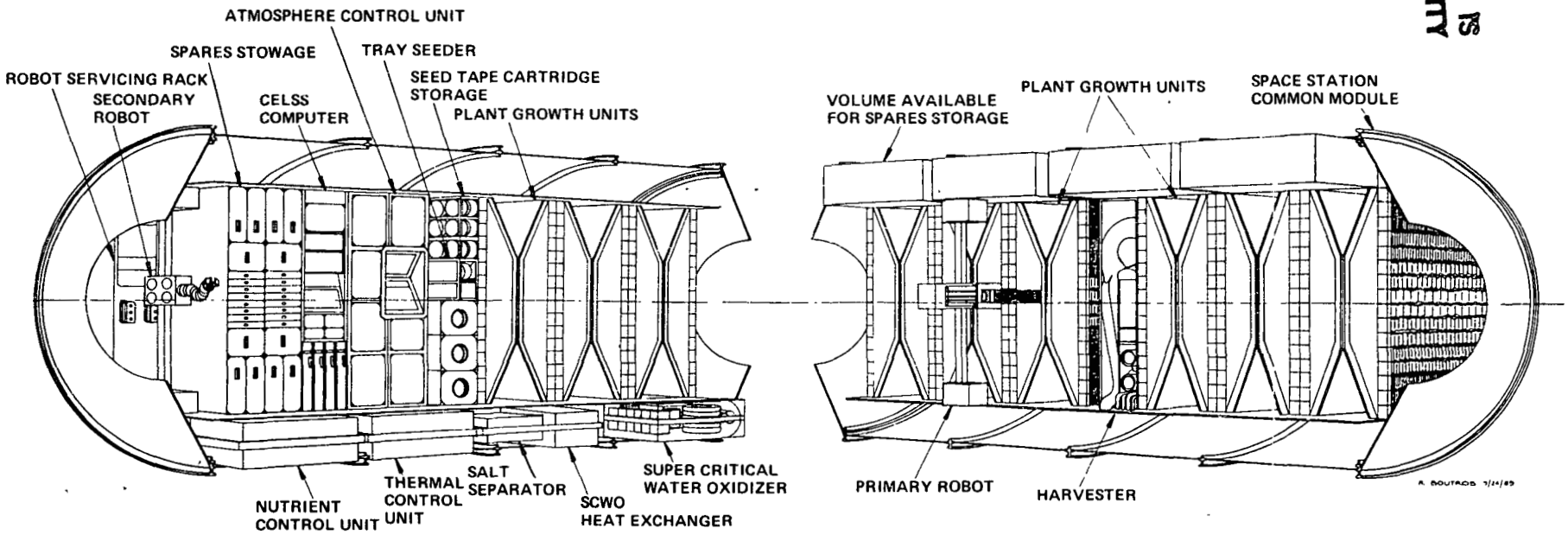

Figure 6. Layout of an operational CELSS module from a Boeing study. Operations are fully automated with robots moving plant trays from the seeding station to the growth chambers and to the harvest equipment. A super critical water oxidizer (SCWO) processes small batches of waste biomass at high speed.

of aromatic trace contaminants from the growing of some flavoring herbs.

A major consideration in the design of a CELSS module is waste processing, particularly of the large amount of inedible plant biomass. A non-microbiological process may be most efficient. A promising unit for the waste processing subsystem is a Super Critical Water Oxidation (SCWO) reactor. Water is heated up to 700oC, pressurized with air or oxygen to 3000 psi and injected into small batches of ground waste slurry, which is then oxidized within seconds into carbon dioxide, nitrogen, water, and mineral salts. Plant nutrients may then be reclaimed and recycled.

When subsystem units for food production, waste regeneration, computer control and automation are finally linked to outfit an operational CELSS module, it may look somewhat like the artist's conception in Figure 6. This accomplishment may be only a decade away with appropriate, continuous research support, and the pooling of information among biologists and bioengineers working in related projects.

The U.S. announcement to establish a Space Station provides an opportunity for cooperation with other national space agencies, laboratories and industries. Such opportunities are especially promising for CELSS research because of the expertise that exists around the world in related ventures. The most efficient development of CELSS would probably result from careful pooling of international resources. If stable and productive materially-closed ecosystems can be made to function on Earth, they could probably be made to work in space. Increased international cooperation will enhance the progressive, logical development of bioregenerative life support systems for use in future space missions.

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16 Abstract

The Controlled Ecological Life Support Systems (CELSS) Program sponsored three meetings during the period July 16 to 19, 1985. These were (1) a Symposium on Cooperation in Biological Space Research, held at Ames Research Center, Moffett Field, CA; (2) a program on International CELSS in San Francisco, CA, sponsored by the Society of Automotive Engineers; and (3) a Workshop on Current CELSS Research, held at Ames. The meetings were attended by a variety of scientists from the United States, Canada, Great Britain, France, Japan, Sweden, and Germany. These Proceedings summarize the comments of the Symposium speakers, reprint papers that were presented at the International CELSS program, and publish the 37 papers contributed in the CELSS Workshop.

The thrust of the presentations and discussions was the general applicability of biologically based regenerative life support systems, and included consideration of international involvement in the scientific research, technology development, engineering and flight testing of the concepts and components of a bioregenerative life support system. Technical details of basic research in plant physiology, food processing, waste processing, plant disease problems, algal growth and physiology, flight system design, bioreactor processing, and growth chamber design and construction are presented in the papers included in the Proceedings.

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